JOURNAL OF AGRICULTURAL RESEARCH

VOL. 70 WASHINGTON, D. C., MARCH 15, 1945 NO. 6

TOTAL AND FREE AMYLASE CONTENT OF DORMANT CEREALS AND RELATED SEEDS[1]

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INTRODUCTION

Studies of the diastatic power of dormant cereal seeds have been made by a number of investigators, mostly within recent times. These studies have been concerned largely with barley because of the possible correlation between the amylase content of the dormant seeds and that of the malt.

Most of the past studies have dealt with three problems: (1) The role of variety in determining the variation in the amylase content of barleys; (2) the mechanism of stimulation of the diastatic activity by natural substances developed in the seeds during germination or by extraneous substances added to the dormant grains or malts during the extraction period; (3) the nature of the amylase of dormant seeds and malts.

Myrbäck (*29*)[2] after extensive study came to the conclusion that when wide allowances are made for environmental factors such as climate, rainfall, soil, and methods of cultivation, variety is definitely one of the factors responsible for the variation in the amylase content of barley. Chrzaszcz and Sawicki (*14*), on the other hand, claim that extensive studies carried out by them showed no variation in the amylase content of barley that could be attributed to the influence of variety. Myrbäck's views seem to be supported by Mangels (*28*), Dickson and associates (*16*), Hills and Bailey (*19*), and Sallans and Anderson (*33*).

The mechanism of activation of the diastatic power in cereals and malts, studied first by Ford and Guthrie (*17*), has been the subject of wide discussion. Two recent views on this subject are expressed by Myrbäck and Örtenblad (*30*) and by Chrzaszcz and Janicki (*7*, *8*). According to Myrbäck and Örtenblad the amylase in cereals is combined with proteins and the activator, which is a proteinase, splits up the proteins and sets the amylase free. According to Chrzaszcz and Janicki there are present in the seeds inhibitors (sisto-substances) which interfere with the functioning of the amylase. The activators (the eleuto-substances) counteract the effect of these natural inhibitors. Added extraneous activators act as eleuto-substances.

The nature of the amylase found in dormant seed has also been a subject of wide study. It is universally recognized that the amylase in dormant seeds is mostly beta-amylase. There is, however, a disagreement among investigators as to whether or not the alpha-amylase, which is found in appreciable quantities in barley malt, is

[1] Received for publication, May 21, 1943.

[2] Italic numbers in parentheses refer to Literature Cited p. 199

not also found in small quantities in dormant seeds. Nordh and Ohlsson (*31*), Lüers and Rümmler (*27*), and Chrzaszcz and Janicki (*9*) believe that alpha-amylase is entirely absent from dormant seeds. On the other hand, Józsa and Gore (*24*), Sandstedt and associates (*34*), and Shellenberger and Bailey (*35*) claim that it is present in dormant seeds. Hills and Bailey (*19*) recently recorded quantitative measurements of small amounts of alpha-amylase in several varieties of barley.

In the present investigation the amylase content of dormant seeds was studied as a property of cereals in general, and not with regard to their malting properties only. The amylase content of cereals used for bread making, such as wheat and rye, may prove to be one of the factors that determine the baking qualities of their flours. The amylase content of other seeds may be a factor in determining their suitability as flour substitutes and in developing the baking procedure to be adopted. A more comprehensive knowledge of the amylase content of seeds may also throw light on the dormancy of winter grains and the problem of vernalization.

MATERIALS AND METHODS

The principal cereals studied included four varieties each of barley, wheat, rye, and oats, two varieties of corn, and one variety of rice. By way of comparison one variety of buckwheat and two varieties each of soybeans, sorghums, and cowpeas were also studied. All seeds were of known origin and were obtained from the Bureau of Plant Industry, Soils, and Agricultural Engineering, United States Department of Agriculture. The ordinary white flour used was obtained on the open market.

The extraction of the free amylase was carried out according to the approximate procedure of the British Institute of Brewing (*20*). Two grams [3] of the ground dormant seeds were weighed out into 125-ml. Erlenmeyer flasks. Sixty milliliters of distilled water were added to each flask and the contents stirred with a glass rod. Ten drops of toluene were added to each flask. The flasks were then stoppered and allowed to stand in a constant-temperature room at 70° F. and shaken every half hour. The extraction of the total amylase was carried out in the same way as the free amylase except that 60 ml. of a half-percent solution of papain were used instead of distilled water. The extractions of the amylase from barley and rye, shown in tables 1 and 3, were carried out in duplicate. The results obtained from the duplicate extractions agreed reasonably well. The results recorded in the other tables were obtained from single extractions. Frequent repetitions, however, were made to check the results.

For the determination of the diastatic power [4] of the extracts 3 ml. of the supernatant clear liquid were withdrawn with a pipette from each Erlenmeyer flask and placed in a corresponding 200-ml. volumetric flask containing 100 ml. of a 2-percent buffered solution of soluble starch. This was repeated at various intervals such as 3, 24, 48, and 72 hours. The contents of the volumetric flask were well shaken and

[3] For malt extractions 1-gm. charges were used.

[4] The terms "diastatic power," "amylase," "diastase," and "saccharifying power" are used interchangeably. The terms "free" and "total" designating respectively the amylase extracted with distilled water and with the aid of papain, were first used by Myrbäck (*29*).

allowed to stand for 1 hour at 70° F. in a constant-temperature room. Six milliliters of a half normal solution of sodium hydroxide were then added to each volumetric flask in order to stop the saccharifying action. The volumetric flasks were then allowed to regain room temperature and made up to volume.

The maltose determinations were made by the Lane and Eynon method (*25*). With material which had a low amylase content, the excess of Fehling's solution after the addition of 25 ml. of the hydrolyzed starch solution was titrated back with a standardized half-percent solution of dextrose. The results were converted into degrees Lintner and represent averages of duplicate determinations.

DIASTATIC POWER OF DORMANT SEEDS

The ground materials were extracted as just described above and the total and free amylase strength for every extraction period was determined, the free amylase being also computed in percentages of total diastatic power. The results are given in tables 1 to 6.

Barley, wheat, and rye possess an appreciable diastatic power (tables 1, 2, and 3). The extraction of free amylase was not complete after 24 hours, the usual time limit used by numerous investigators. In these experiments maximum extraction was not reached until about 3 days. This is true also for total amylase except for wheat, in which the maximum extraction was reached in 3 hours or less. Rye and wheat seem to contain more total and free amylase than barley. Rye has a higher free diastatic power than wheat, but the total diastatic strength of wheat is higher than that of rye.

TABLE 1.—*Development of total and free diastatic power in degrees Lintner in 4 varieties of dormant barley during different periods of extraction*

Variety	Diastatic power for extraction period of—											
	3 hours			26 hours			74 hours			98 hours		
	Total	Free	Ratio of free to total	Total	Free	Ratio of free to total	Total	Free	Ratio of free to total	Total	Free	Ratio of free to total
	°*L.*	°*L.*	*Percent*	°*L.*	°*L.*	*Percent*	°*L.*	°*L.*	*Percent*	°*L.*	°*L.*	*Percent*
Wisconsin Winter	77.2	43.0	55.7	84.0	49.8	59.3	90.5	60.6	67.0	88.5	60.3	68.1
Tennessee Winter	71.1	40.1	56.4	75.2	46.7	62.1	85.9	56.3	65.5	79.4	55.6	70.0
Tennessee Beardless No. 6 (C. I.[1] 2746)	49.6	20.1	40.5	58.5	27.4	46.8	68.1	36.1	53.0	63.7	35.6	55.9
Esaw	59.9	32.8	54.8	66.5	39.6	59.6	71.9	46.7	65.0	70.4	44.8	63.6

[1] C. I. refers to accession number of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering.

The response to the action of papain is judged from the percentages of free amylase calculated in terms of total amylase (column 3 for each extraction period). A high percentage of free amylase indicates a low response to papain and vice versa. The response is about the same for barley and wheat but considerably less for rye. In accordance with Myrbäck's theory,[5] which is followed in this discussion and in the interpretation of results, this means either that the amylase in

[5] See Introduction.

rye is less bound to the proteins than in wheat and barley or that rye possesses more natural proteinases [6] than do wheat and barley.

TABLE 2.—*Development of total and free diastatic power in degrees Lintner in 4 varieties of dormant wheat during different periods of extraction*

Variety	Diastatic power for extraction period of—								
	3 hours			27 hours			49 hours		
	Total	Free	Ratio of free to total	Total	Free	Ratio of free to total	Total	Free	Ratio of free to total
	°*L.*	°*L.*	*Percent*	°*L.*	°*L.*	*Percent*	°*L.*	°*L.*	*Percent*
Federation	104.1	55.5	53.3	102.1	55.5	54.4	103.0	59.2	57.5
Marquis	116.2	59.2	51.0	115.0	57.8	50.3	112.2	59.5	53.0
Baart	110.0	52.9	48.1	108.8	50.3	46.2	106.2	52.6	49.5
Golden	90.1	44.8	49.7	90.0	43.5	48.4	89.3	44.6	49.9

Variety	Diastatic power for extraction period of—							
	73 hours			97 hours			122 hours	
	Total	Free	Ratio of free to total	Total	Free	Ratio of free to total	Total	Free
	°*L.*	°*L.*	*Percent*	°*L.*	°*L.*	*Percent*	°*L.*	°*L.*
Federation	103.0	62.1	60.3	102.0	64.1	62.8	------	64.9
Marquis	110.0	62.9	57.5	113.7	65.4	57.5	------	64.5
Baart	106.2	55.9	52.6	106.2	57.8	57.6	------	57.5
Golden	87.0	46.7	53.7	89.3	48.8	54.7	------	47.6

TABLE 3.—*Development of total and free diastatic power in degrees Lintner in 4 varieties of dormant rye during different periods of extraction*

Variety	Diastatic power for extraction period of—											
	3 hours			27 hours			51 hours			75 hours		
	Total	Free	Ratio of free to total	Total	Free	Ratio of free to total	Total	Free	Ratio of free to total	Total	Free	Ratio of free to total
	°*L.*	°*L.*	*Percent*	°*L.*	°*L.*	*Percent*	°*L.*	°*L.*	*Percent*	°*L.*	°*L.*	*Percent*
Dakold	93.4	71.9	77.1	92.4	76.6	82.9	99.0	83.0	83.8	104.7	85.8	82.0
Star	89.3	70.9	79.4	94.8	76.9	81.1	96.6	82.2	85.5	103.1	86.6	84.0
Rosen	110.5	89.7	81.2	112.2	97.1	86.5	114.2	102.0	89.3	109.4	103.6	94.6
Abruzzi	85.0	65.8	77.4	88.5	71.4	80.7	89.5	77.0	86.0	94.4	82.9	87.8

The four varieties of each barley, wheat, and rye used show a marked variation in diastatic power. They were all grown in the same locality and in the same crop year and the results seem to support Myrbäck's view that variety is a factor in determining diastatic-power differences in cereals.

Dormant oats, corn, and rice (tables 4 and 5) have very little diastatic power. Their total and free diastatic power is about the same, showing that it is not lack of proteinases that is responsible for their low amylase content. Oats seem to be somewhat higher in diastatic

[6] The term "proteinase" as used in this paper includes all natural substances present in biological materials which stimulate, or liberate, amylase.

power than either corn or rice and also show a greater variation between varieties.

TABLE 4.—*Development of total and free diastatic power*[1] *in degrees Lintner in 4 varieties of dormant oats during different periods of extraction*

Variety	Diastatic power for duration of extraction period of—					
	3 hours		24 hours		48 hours	
	Total	Free	Total	Free	Total	Free
	° *L.*	° *L.*	° *L.*	° *L.*	° *L.*	° *L.*
Fulghum	3.7	4.5	3.7	4.5	3.7	3.7
Iogold	1.5	1.5	2.2	2.2	2.2	2.2
Lee	1.5	2.2	2.2	2.2	1.5	1.5
Winter Turf	.7	.7	1.5	1.5	2.2	3.0

[1] Corrected for blank values which were about 2.3° L.

TABLE 5.—*Development of total and free diastatic power*[1] *in degrees Lintner in dormant corn and rice during different periods of extraction*

Cereal	Variety	Diastatic power for extraction period of—			
		3 hours		24 hours	
		Total	Free	Total	Free
		° *L.*	° *L.*	° *L.*	° *L.*
Yellow corn	Long-husked Krug	0.7	none	0.7	none
White corn	Thompson Prolific	.7	none	.7	none
Rough rice	Nira	1.5	1.5	.7	none
Brown rice	do	.7	none	.7	none

[1] Corrected for blank values, which were about 2.3° L.

TABLE 6.—*Development of total and free diastatic power*[1] *in degrees Lintner in dormant buckwheat, soybeans, sorghums, and cowpeas during different periods of extraction*

Material	Variety	Diastatic power for extraction period of—			
		3 hours		24 hours	
		Total	Free	Total	Free
		° *L.*	° *L.*	° *L.*	° *L.*
Buckwheat	Silverhull	1.5	1.5	2.2	2.2
Soybeans	Easycook	78.4	78.4		
Do	Rokusun	57.9	58.3	56.5	57.6
Sorghum	Milo	.7	3.0		
Do	Sharon Kafir	.7	.7		
Cowpeas	Brabham	.7	3.0		
Do	Iron	1.5	.7		

[1] Corrected for blank values, which were about 2.3° L.

Of the sorghums and the noncereal seeds (table 6) only the soybeans possess an appreciable diastatic power. Their maximum saccharifying power was developed in 3 hours or less and showed no response to papain, suggesting that the soybeans contain sufficient natural proteinase for the liberation of their saccharifying power. The fact that soybeans are known to be rich in proteinases would seem to support

Myrbäck's theory of the mechanism of liberation of amylase in dormant cereals.

EFFECT OF LACTIC ACID ON LIBERATION OF DIASTATIC POWER

Gőmőry (*18*) used lactic acid as a means of increasing the diastatic power of flour. In the present investigation the effects of lactic acid and papain on the development of diastatic power in barley, wheat, and rye were compared (table 7). The concentration of lactic acid

TABLE 7.—*Effect of lactic acid with and without papain on the development of diastatic power in dormant barley, wheat, and rye during different periods of extraction*

Cereal	Variety	Extractor	Diastatic power for extraction period of—				
			3 hours	26 hours	50 hours	74 hours	98 hours
			°*L.*	°*L.*	°*L.*	°*L.*	°*L.*
Barley	Wisconsin Winter	Water	34.5	37.6	46.9	50.8	51.0
		Papain [1]	66.7	69.0	76.4	79.4	77.5
		Lactic acid [2]	17.5	19.0	27.3	31.4	31.4
		Papain + lactic acid	63.7	68.5	73.5	77.5	75.8
Wheat	Federation	Water	55.8	55.8	55.8	59.8	61.4
		Papain	102.0	105.2	104.1	104.1	104.1
		Lactic acid	63.3	72.0	82.6	88.5	89.2
		Papain + lactic acid	103.0	106.2	103.1	104.1	105.2
Rye	Rosen	Water	87.7	93.4	100.0	103.0	103.1
		Papain	111.1	112.2	112.2	113.6	112.2
		Lactic acid	79.4	86.2	93.5	101.0	100.0
		Papain + lactic acid	104.1	110.2	111.1	116.2	112.2

[1] 1/2-percent solution.
[2] 2 cc. of 1-percent lactic acid solution added to the water (60 cc.).

used was 1 percent on the basis of the cereal charges used (the optimum concentration in Gömöry's experiments) or 0.033 percent on the basis of the extracting solution.

There were striking differences in the response of the three cereals to the lactic acid extraction. It definitely increased the diastatic strength of wheat and definitely decreased that of barley. In rye the lactic acid decreased somewhat the diastatic strength in the shorter periods of extraction but had no effect in the longer periods. It had no effect when used together with papain.

The fact that the lactic acid acted differently on the three cereals indicates that its action in increasing the diastatic power of wheat was not a direct hydrolyzing effect. Its different action on barley and wheat, however, may be explained by its effect on the hydrogen-ion concentration of their extracts.

The pH of the distilled water extracts was about 6.5 for wheat and about 5.7 for barley. Balls and Hale (*3*) found that the proteinases of wheat had two optima of digestion, at pH 3.3 and pH 5.0, and that those of barley had a broad pH optimum of digestion from pH 4.0 to 7.0. Accordingly, the pH of the water extracts of wheat was definitely above the pH optima and the pH of the water extracts of barley was right at the optimum. The pH of the lactic acid solution (0.033 percent) used for extraction was 2.90 which is, according to the data of Balls and Hale, close to the lower optimum of digestion for the wheat proteinases but considerably below the optimum for barley. The resultant pH of the lactic acid extracts (in special tests) increased rapidly up to about 3 hours, after which it became about stationary,

namely, pH 4.52 for both the barley and the wheat extracts. This pH is close to the pH optimum of Balls and Hale for wheat, and the increase in diastatic power of wheat caused by lactic acid can be consistently explained by its effect both on the initial and final pH of the extracts. But the final pH of the extracts is also close to the lower end of the broad optimum of digestion for barley mentioned above and therefore could not apparently serve as the cause of the decrease in diastatic activity produced by lactic acid in the barley extracts. However, it is possible that the decrease occurred before the pH of the extract reached its final value. Furthermore, when the data of Balls and Hale (*3, table 2*) are examined more closely, a possible break in the broad pH optimum of proteinase digestion of barley suggests itself at pH 4.50, which is practically identical with the final pH (4.52) of the barley and wheat extracts obtained on the addition of the lactic acid solution.

The results obtained in this experiment, and perhaps also those of Balls and Hale, are not to be viewed as definite values but merely as data showing certain tendencies. This fact, however, should not detract from the plausibility of the explanation offered.

The lactic acid had no effect on the extraction of amylase with papain, probably because the latter was used in large excess. Attention is called to the fact that the increase in the diastatic power caused by lactic acid is not as large as that caused by papain.

EFFECT OF TOLUENE AND MICRO-ORGANISMS ON THE DEVELOPMENT OF DIASTATIC POWER

In the study of the diastatic power of dormant seeds toluene is generally added to prevent microbiological activity. It is important to know whether toluene itself exerts any stimulating or inhibiting influence on the development of diastatic power. It is also of interest to study the effect of the mixed microflora coming from the room atmosphere or from the seeds on the development of diastatic power when its activity is not inhibited by toluene.

In this experiment in addition to ground barley, wheat, and rye, ordinary white wheat flour was used. The results (table 8) show that the use of toluene in water extracts had no effect on rye and only a slight effect on barley but that it had a pronounced depressing effect on the diastatic activity of ground whole wheat and flour.

The fact that when papain was used toluene had no effect on the development of the diastatic power in wheat and flour and also the fact that it had no influence on its development in rye even when no papain was used, tends to show that toluene itself has no direct effect on the development of diastatic power. The depression of the diastatic power in wheat and flour caused by the addition of toluene was evidently due to its inhibiting effect on the growth of micro-organisms.

Jørgensen (*21*) stated that toluene interfered with the depression in the nitrogen solubility of flour caused by the bread improvers—potassium bromate and potassium iodate. The solubility of flour nitrogen, according to Jørgensen, is due to the action of proteinases, similar to the liberation of amylase according to Myrbäck. However on examining closely Jørgensen's data, it is apparent that the depression in nitrogen solubility caused by toluene alone is of about the

same magnitude as that caused by potassium iodate alone. It is possible, therefore, that the solubility of the nitrogen present in the flour was partly due to microbiological activity and that the effect of toluene was to inhibit this activity.

TABLE 8.—*Effects of toluene and papain on the development of diastatic power in dormant barley, wheat, and rye and in ordinary wheat flour*

Material	Variety	Extractor [1]	Diastatic power for extraction period of—			
			3 hours	27 hours	51 hours	72 hours
			°*L.*	°*L.*	°*L.*	°*L.*
Barley	Wisconsin Winter	Water	31.2	35.6	48.5	49.2
		Water+toluene	30.4	32.4	45.4	45.0
		Papain solution	58.8	63.7	76.3	74.1
		Papain+toluene	58.8	61.7	73.5	73.5
Wheat	Federation	Water	52.6	52.4	86.2	94.3
		Water+toluene	54.8	52.4	56.5	57.5
		Papain solution	104.1	103.1	106.2	104.1
		Papain+toluene	102.0	103.1	106.2	105.2
Rye	Rosen	Water	85.4	90.0	100.0	99.0
		Water+toluene	85.4	87.7	100.0	99.0
		Papain solution	92.6	103.1	113.7	108.5
		Papain+toluene	102.0	108.7	114.9	112.2
Flour		Water	26.5	25.5	96.1	111.1
		Water+toluene	26.2	24.7	25.0	24.2
		Papain solution	90.9	103.1	119.0	119.0
		Papain+toluene	104.1	102.0	119.0	120.8

[1] Quantity of toluene added, 10 drops.

In ground wheat and in flour the increase in diastatic power caused by microbiological activity was almost as high as that caused by papain. The difference in response to microbiological action between wheat and flour on the one hand and barley and rye on the other is striking (table 8).

DISTRIBUTION OF PROTEINASES AND DIASTATIC POWER IN WHEAT GERM, BRAN, AND FLOUR

The results given in table 8 show also marked differences in the development of diastatic power between ground whole wheat and white flour. With papain or in water without toluene (subject to microbic action) the diastatic power of the flour after 3 days is consistently higher than that of the ground wheat; with toluene or without papain the ground wheat is more than twice as high in diastatic power as the flour. In accordance with Myrbäck's theory whole wheat is therefore richer in active proteinases than flour but poorer in amylase.

To determine whether such is actually the case, bran and germ were each added to flour to the extent of 10 percent of the charges and their effect on the development of diastatic activity of the flour was determined (table 9). This experiment, as well as all other experiments unless otherwise stated, was carried out with the addition of toluene. The flour, germ, and bran were all commercial products but were not obtained from the milling of the same wheat. The Lintner values were corrected for the values of the added bran and germ (free diastatic power, table 10.) The results show that the addition of germ increased markedly the diastatic power of the flour while the addition of bran had but little effect. This tends to show that the proteinases in wheat are largely concentrated in the germ.

Further tests were made by determining the respective response of germ and bran to the added proteinase papain (table 10). It is seen from the percentage recovery of total diastatic power that the bran responded to the papain considerably more than did the germ, indicating that the latter has more natural proteinases than the former. The results also show that both the bran and the germ were lower in total diastatic power than either the ground whole wheat or the flour (table 8), which is in harmony with the fact that flour is higher in total diastatic power than whole wheat.

TABLE 9.—*Effect of addition of 10 percent of wheat bran and wheat germ on the development of diastatic power in flour (duration of extraction 3 hours)*

Material added	Diastatic power		
	Found	Calculated	Increase
	°*L.*	°*L.*	°*L.*
None	27.0		
Germ	44.4	31.1	13.3
Bran	32.2	29.9	2.3

TABLE 10.—*Development of total and free diastatic power in degrees Lintner in wheat bran and wheat germ (duration of extraction 3 hours)*

Material	Diastatic power		
	Total	Free	Ratio of free to total
	°*L.*	°*L.*	*Percent*
Germ	84.0	68.5	81.5
Bran	95.2	56.2	59.0

THE RELATIVE PROTEINASE CONTENT OF VARIOUS SEEDS

The previous experiments have shown that two conditions are necessary for the development of diastatic power in dormant seeds; namely, the presence of amylase and the presence of a stimulating principle which, according to Myrbäck, consists of proteinases.

Some of the seeds tested (tables 4, 5, and 6) failed to develop any appreciable diastatic power, but they also failed to respond to the added proteinase papain. This would tend to show that these seeds are actually lacking in amylase. It was desired to test the various seeds studied for the proteinases responsible for the liberation of amylase. Flour, which is relatively poor in active proteinase but rich in amylase, served as a good testing medium. Accordingly, the seeds studied were added to flour to the extent of 10 percent of the total charges (1.8 gm. of flour and 0.2 gm. of the respective ground seeds) and their effect on the development of the diastatic power was determined.

The results (table 11) show that the amylase content and proteinase content of dormant seeds are not correlated. Barley and rye with an appreciable amylase content have the lowest proteinase content of all seeds studied, while soybeans, also with an appreciable amylase content, have the highest proteinase content. All of the seeds studied which had a low amylase content or none at all showed a relatively high proteinase content, particularly the cowpeas.

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TABLE 11.—*Effect of addition of 10 percent of various seeds on the development of diastatic power in flour (duration of extraction 3 hours)*

Ground seed added	Diastatic power		
	Found	Calculated	Increase
	°*L.*	°*L.*	*Percent*
None	26.0		
Barley	31.3	27.4	14.2
Rye	39.7	32.4	22.5
Oats	42.4	23.5	80.4
Corn, yellow	36.4	23.4	55.6
Corn, white	35.2	23.4	50.4
Rice	37.6	23.5	60.0
Buckwheat	42.0	23.5	78.7
Soybeans	76.3	31.2	144.5
Sorghum	43.4	23.7	83.1
Cowpeas	52.1	23.7	119.8

EFFECT OF SODIUM CHLORIDE ON THE DEVELOPMENT OF DIASTATIC POWER IN CEREALS

The total diastatic power of a plant material in this investigation was determined with the aid of papain. Other proteinases also liberate amylase. Thus Chrzaszcz and Janicki (*10, 11*) found that trypsin and rennet liberated amylase in cereals, while pepsin had no effect. It has been found in this investigation, however, that pepsin liberates amylase in flour.

Since proteinases have a solvent action on proteins, it was desired to determine the effect on the liberation of amylase of an inorganic salt such as sodium chloride which also has a solvent action on protein nitrogen. Chrzaszcz and Janicki (*13*) tested the effect of this salt on ripening wheats and obtained slight increases in saccharifying power. Ford and Guthrie (*17*) obtained increases of saccharifying power in ground barley with potassium chloride.

In this investigation the effect of a 1-percent sodium chloride solution was tested on barley, wheat, rye, and oats. The response of these cereals to this treatment (table 12) showed a marked variation.

The use of sodium chloride resulted in a distinct increase in the diastatic power of the wheat, in a marked decrease in the diastatic power of the barley, and in a small decrease in the diastatic power of the rye. While there was an apparent increase in the diastatic power of the oats, the values involved are too small to indicate the tendency clearly.

The effect of sodium chloride on diastatic power was tested on a few other varieties of wheat (Poole, Fultz, American Banner, Purplestraw) and barley (Mekano Wase and Esaw). The results reported (table 12) for wheat were in a general way corroborated, but those reported for barley were not sustained. It is possible that the effect of sodium chloride on the diastatic power of barley (and perhaps also of the other grains) is a varietal characteristic. Anderson and Ayre (*2*) found that the variation in salt-soluble nitrogen is a varietal characteristic of barley. It is possible that there is a correlation between the effect of sodium chloride on the nitrogen solubility of seeds and its effect on their diastatic power.

TABLE 12.—*Effect of sodium chloride on the development of diastatic power*[1] *in dormant barley, wheat, rye, and oats*

Cereal	Extractor	Diastatic power for extraction period of—		
		4 hours	24 hours	72 hours
		°*L.*	°*L.*	°*L.*
Barley	Water	40.7	47.5	58.3
	Sodium chloride solution [2]	24.5	28.5	32.8
Wheat	Water	53.2	53.2	59.8
	Sodium chloride solution [2]	75.2	80.3	77.7
Rye	Water	87.4	94.8	101.3
	Sodium chloride solution [2]	83.9	92.1	92.9
Oats	Water	.7	1.5	
	Sodium chloride solution [2]	1.5	2.2	2.2

[1] Corrected for blank values which were about 2.3° L.
[2] 1 percent.

Attention is called to the fact that the increase in the diastatic power of wheat caused by sodium chloride (table 12) is less than that caused by papain (table 2).

EFFECT OF PAPAIN ON THE DEVELOPMENT OF DIASTATIC POWER IN FLOUR

Flour, as was shown above, is rich in total amylase and poor in substances which set this amylase free. It could, therefore, serve as an appropriate medium for studying the effect of different activating agents on the liberation of amylase. The effect of various concentrations of papain on the development of diastatic power in flour was studied first.

TABLE 13.—*Effect of various concentrations of papain on the development of diastatic power in flour during different periods of extraction*

Concentration of papain solution	Diastatic power for extraction period of—				
	3 hours	24 hours	48 hours	72 hours	144 hours
Percent	°*L.*	°*L.*	°*L.*	°*L.*	°*L.*
None	26.5	25.6	24.6	23.3	
0.5	120.5	120.5			
.25	120.5	122.0			
.10	122.0	119.0			
.050	119.0	119.0			
.025	117.7	117.7			
.010	111.1	117.7			
.005	89.3	117.7	119.0		
.0025	66.7	85.5	109.0	115.0	120.5
.0010	54.3	86.2	89.3	104.2	116.2
.0005	43.5	55.6	59.2	62.2	72.0

The results (table 13) show that between 0.5 percent and 0.025 percent, the concentration of the papain solution had no effect on the magnitude of the developed diastatic power in 3 hours. The variation in the Lintner values obtained between the limits of these concentrations are to be considered as mere fluctuations in view of the fact that there was no further increase in diastatic power in 24 hours over the corresponding values obtained in 3 hours. Evidently all the concentrations above 0.025 percent were in excess of what was necessary to

liberate the maximum saccharifying power in 3 hours; with the concentrations of papain lower than 0.025 percent the rate of increase in diastatic power was slowed up. With the concentration of 0.0025 percent the maximum saccharifying power was obtained in between 72 and 144 hours. With the lowest concentration the maximum saccharifying power had not been reached after 144 hours when the experiment was discontinued.

Attention is called to the gradual decrease in the free amylase content of the control. The values involved are small but they seem to be consistent, as will be seen in the following experiments.

EFFECT OF PAPAIN ON THE LOAF VOLUME OF BREAD

The theory of Myrbäck and other workers with regard to the development of diastatic power in dormant cereals is completely analogous to the theory of Jørgensen and others (*21*, *5*) with regard to the mechanism of the action of bread improvers. The development of free amylase in dormant cereals, according to the one theory, is due to the action of natural proteinases which liberate the amylase by attacking the proteins to which it is bound; added proteinases complete this liberation. The failure of some flours to yield bread of a good loaf volume, according to the other theory, is due to an excess of natural proteinases which weaken the proteins; the bread improvers inhibit the action of these proteinases. By adding relatively large quantities of papain Balls and Hale (*4*) obtained completely collapsed breads.

It was therefore desired to test the effect of the papain concentrations used in the previous experiment (table 13) on the loaf volume of breads obtained with ordinary white flour.

The baking tests were carried out according to the standard procedure of the American Association of Cereal Chemists (*1*). The concentrations of papain (table 14) are given on the basis of the water added (60 cc. of water to 100 gm. of flour). The transverse and longitudinal measurements, as well as the abstract figures representing the products of these respective measurements, give a good idea of the actual gradation in the loaf volume of these breads. There was good correlation between the concentration of papain and the gradation in the loaf volume of the breads. The decrease in volume, as compared with the control loaf, occurred in all except the lowest concentration of papain.[7]

The fact that both the liberation of amylase and the decrease in loaf volume are consistently affected by the concentration of an added proteinase seems to lend mutual support to the two analogous theories. It also seems that the same kind of proteins are involved in the two phenomena. This in turn suggests that only part of the proteins are involved in the liberation of amylase or in the decrease of loaf volume of bread. Such a possibility has indeed been advanced by Myrbäck and Örtenblad (*30*) with regard to the proteins involved in the liberation of amylase, in their controversy with Chrzaszcz and Janicki (*12*). These authors contended that if Myrbäck's theory were true there ought to be a correlation between increased amylase activity and the solubility of protein nitrogen, which, as they found, was not the case. According to Myrbäck and Örtenblad, however, it was not to be

[7] The effect of papain on the loaf volume of bread was discussed more fully in a previous paper (*15*).

expected that the presumably quantitatively small mass of the amylase substance would be bound to all the proteins; the solubility, therefore, of the correspondingly small portion of the proteins to which the amylase may have been bound could not be detected in the determination of the general solubility of the protein nitrogen.

TABLE 14.—*Effect of various concentrations of papain on the loaf volume of bread*

Concentration of papain solution	Dimensions of loaf		
	Transverse circumference	Longitudinal circumference	Transverse [1] multiplied by longitudinal circumference
Percent	*Inches*	*Inches*	
None	12.8	16.0	205
0.5	9.6	12.6	121
.25	9.8	12.5	122
.125	10.4	13.3	138
.062	10.8	13.8	149
.010	10.7	14.3	153
.005	11.1	14.3	159
.002	11.9	15.3	182
.001	12.5	15.7	196
.0005	12.7	16.1	205

[1] These figures are numerical expressions of the relative volume of the loaves.

EFFECT OF CYSTEINE HYDROCHLORIDE ON THE DEVELOPMENT OF DIASTATIC POWER IN FLOUR

Balls and Hale (*4*) separated from wheat a partially purified proteinase of the papainase type. This proteinase was activated by cysteine hydrochloride, potassium cyanide, and hydrogen sulfide. In following up the analogy between the theory with regard to bread improvers and the theory with regard to the liberation of amylase by proteinases, the effect of cysteine on the development of the diastatic power in flour was tested.

TABLE 15.—*Effect of cysteine hydrochloride on the development of diastatic power in flour during different periods of extraction*

Concentration of cysteine hydrochloride	Diastatic power for extraction period of—	
	3 hours	24 hours
Percent	° *L.*	° *L.*
None	22.2	22.2
0.3	116.3	120.5
.15	115.0	116.3
.075	96.2	103.1
.037	74.6	80.0

It is shown (table 15) that cysteine hydrochloride has a distinctly stimulating effect on the diastatic activity of the flour and that this stimulation is related to the concentration of the cysteine. The analogy between the two theories is thus sustained.

Much higher concentrations of cysteine than of papain (table 13) were required to produce corresponding stimulating effects on the liberation of amylase. This may be analogous to the results of Balls

and Hale (*5*) who found that relatively large quantities of cysteine hydrochloride were required to complete the dispersion of gluten.

The suggestion made by Balls and Hale (*5*) that cysteine not only serves to stimulate the proteinase but that it also affects the proteins directly, may likewise apply to the stimulation of diastatic activity by this activator. This suggests the possibility that the apparent proteinase deficiency in flour is in a certain degree an activator deficiency which is corrected by such substances as cysteine.

Snider (*36*) found that the addition of cysteine reduced the extraction time of barley malt from 20 hours to 3.

EFFECT OF POTASSIUM IODATE ON THE DIASTATIC POWER OF FLOUR

According to the theory of bread improvers mentioned above, the action of proteinases is inhibited by chemical oxidants, such as potassium bromate and potassium iodate. Jørgensen (*22*) showed that the water-soluble nitrogen in flour was depressed by potassium bromate and potassium iodate. Balls and Hale (*6*) depressed the activity of the proteinase concentrate separated by them from wheat by persulfate, bromate, and metavanadate.

To further test the analogy between the proteinase theories of bread improvement and liberation of amylase, the effect of various concentrations of potassium iodate on the development of diastatic power was studied.

It is shown (table 16) that both the total depression and the rate of depression of the amylase activity are directly related to the concentration of the iodate. Slight depressions of the amylase activity were caused by such low concentrations as a 0.00001 molecular solution of potassium iodate. Table 14 shows that relatively small concentrations of papain caused an appreciable depression in the loaf volume of bread and it was shown elsewhere (*15*) that appropriate concentrations of potassium iodate overcame this depression. The analogy between the two theories is again sustained.

Read and Haas (*32*), in opposing the theory of bread improvers based on the inhibition of proteinases by oxidants, referred to the fact that it takes much more potassium bromate to produce a depression in soluble nitrogen than the quantity of the oxidant required to produce an improvement in loaf volume. Jørgensen (*23*) endeavored to explain this apparent discrepancy. In the present experiments, however, the concentrations of iodate which produced a noticeable depression in the amylase activity of flour are comparable with those used in overcoming the effect of papain in reducing the loaf volume of bread (*15*). It is not claimed that a high amylase content is the cause of decreasing the loaf volume of bread but that the amylase content may, under certain conditions, be an indicator of proteinase activity. When the amylase content is taken as a criterion, not only does the discrepancy pointed out by Read and Haas disappear but there is also, in general, a consistent relation between the concentration of the iodate and the effect produced.

The small quantities of iodate used again suggest that not all the proteins are involved in the two analogous phenomena under discussion but only a small portion of them. Changes in this small portion would not always result in changes in the total nitrogen

solubility which could be detected by ordinary analysis, as was pointed out above.

TABLE 16.—*Effect of various concentrations of potassium iodate on the development of diastatic power in flour during different periods of extraction* [1]

Molal concentration of potassium iodate	Diastatic power for extraction period of—				
	3 hours	24 hours	48 hours	72 hours	144 hours
	° *L.*	° *L.*	° *L.*	° *L.*	° *L.*
None	23.2	20.8	19.2	19.2	----
0.005	11.3	3.0	3.0	----	----
.0025	14.3	3.8	3.0	----	----
.00125	16.3	5.3	3.8	2.3	----
.00062	20.0	8.3	4.5	2.3	----
.00016	21.4	13.6	9.8	7.6	6.0
.00008	23.1	17.6	13.5	11.3	9.8
.00004	22.7	18.9	16.3	15.1	12.1
.00002	23.2	20.0	19.5	17.1	11.3
.00001	23.6	21.2	20.0	17.9	----
.000005	23.3	21.5	21.4	20.0	----

[1] Part of this table was used in a previous paper (*15, table 5*).

The fact that diastatic activity of the flour continually decreased and that, with some of the lower concentrations of the iodate, the maximum decrease had not been reached in 144 hours shows that the oxidant not only inhibits the liberation of the amylase but also inactivates it or destroys it after it is liberated.

EFFECT OF SODIUM CHLORIDE ON THE DEVELOPMENT OF DIASTATIC POWER IN FLOUR

In a previous experiment the effect of sodium chloride on the development of diastatic activity in cereals was tested (table 12). The results were not clearly defined. In this experiment the effect of sodium chloride was tried on flour which, as was pointed out above, serves as a convenient testing material because it is rich in amylase and deficient in proteinases. Salt is also invariably used in bread making. The experiment was carried on in two parts for different intervals of time and with different samples of flour.

Table 17 shows that sodium chloride has a distinctly stimulating effect on the development of diastatic activity and that this effect is directly related to the concentration. As in the case of cysteine, comparatively high concentrations of salt are required to produce the effect and, therefore, it is still a question whether the effect of the salt is on the flour proteinases or directly on the proteins.

The consistent decrease in diastatic activity with time observed in all controls (untreated flours) of the previous experiment (table 13) is sustained in the two controls of this experiment. The same decrease is observed in the two lowest concentrations of salt. This suggests the possibility that there is something in flour, in the nature of the sisto-substances of Chrzaszcz and Janicki (*7, 8*) which has a slightly inactivating effect on amylase. It is possible that sodium chloride, besides its stimulating effect on diastatic activity, also serves as the eleuto-substances of Chrzaszcz and Janicki since this decrease with time is not observed with the concentrations of sodium chloride above 0.025 percent.

TABLE 17.—*Effect of various concentrations of sodium chloride on the development of diastatic power in flour during different periods of extraction*[1]

Part	Concentration of sodium chloride	Diastatic power for extraction period of—			
		3 hours	24 hours	48 hours	72 hours
	Percent	° *L.*	° *L.*	° *L.*	° *L.*
1	None	33.3	29.4	26.7	
	2.0	99.0	104.2	103.1	
	1.0	96.2	100.0	101.1	
	.5	96.2	94.0	93.5	
	.25	86.2	88.5	87.7	
	.10	70.4	70.4	70.9	
2	None	21.1	20.2	19.6	19.7
	.10	66.2	66.2	66.7	66.7
	.05	45.7	45.9	45.9	45.9
	.025	34.6	32.7	30.9	30.9
	.01	26.3	24.0	23.5	23.5

[1] Part of this table was used in a previous paper (*15, table 4*).

To reconcile the decrease in amylase activity after it has been set free, with the proteinase theory, we may assume that the hydrolysis of the protein amylase complex is reversible. The inhibitor or sistosubstance accordingly depresses the proteinase activity, and as a result an equilibrium is established with a lower amylase activity. This is in harmony with the theory of Balls and Hale (*4*) according to which the improvement of the baking quality of some flours with aging is due to the depressed activity of the flour proteinases.

EFFECT OF YEAST ON THE DEVELOPMENT OF DIASTATIC POWER IN FLOUR

When it was found that sodium chloride, an invariable constituent of bread, has a distinctly stimulating effect on the diastatic activity of flour, it was desired to test the effect of yeast, another indispensable constituent of all ordinary bread, on the liberation of amylase in flour. The unidentified microflora ordinarily associated with flour was shown in table 8 to be practically as effective as papain in liberating amylase in flour.

Two concentrations of yeast were used, one which was comparable with bread on the basis of 100 gm. of flour used in the baking of the "small" loaf according to the procedure of the American Association of Cereal Chemists (0.06 gm. yeast per 2 gm. flour and 60 ml. water), and one comparable with bread on the basis of water used (3 gm. yeast per 2 gm. flour and 60 ml. water). The same volume of water (60 ml.) used in the preparation of the small loaf was used for the extraction of amylase in these experiments. The tests with yeast were made with and without toluene.

Table 18 shows that when no toluene was added even the low concentrations of yeast caused a distinct increase in diastatic activity as compared with the control to which toluene was added. However, neither the low nor the high concentration of yeast was as effective in stimulating the diastatic activity of flour as the unidentified microflora associated with flour when its action is not suppressed by toluene (table 8). The probable explanation is that the yeast suppressed the activity of the mixed microflora of the flour and that the former is not as effective in stimulating diastatic activity as the latter.

TABLE 18.—*Effect of yeast on the development of diastatic power in flour during different periods of extraction*

Material	Yeast added	Diastatic power for extraction period of—	
		3 hours	72 hours
	Grams	° *L.*	° *L.*
Flour	0+toluene	26.9	24.3
	.06 yeast only	35.9	97.0
	.06+toluene	31.0	32.4
	3.0 yeast only	94.3	111.1
	3.0+toluene	88.5	108.8
No flour	3.0 yeast only	0	0

This explanation is supported by the fact that the high concentration of yeast used caused practically the same stimulation of diastatic activity in flour with and without the addition of toluene which suppressed microbic activity in the previous experiments. Toluene also practically suppressed the activity of the lower concentration of yeast in this experiment but was ineffective against the higher yeast concentration. The yeast itself had no diastatic power.

The fact that two indispensable ingredients of bread cause a marked increase in the diastatic activity of flour (salt and yeast caused a distinct increase in the diastatic power of dough also) tends to show that the depression in volume of bread resulting from the addition of certain concentrations of papain and other proteolytic enzymes is not due directly to the increased amylase content of the flour caused by these enzymes but to some other factor, probably to the degradation of the proteins. The increase in amylase activity would accordingly serve only as an indicator of proteinase activity. The relation of free amylase activity to total amylase activity in flours should be indicative, in a general way, of their relative proteinase strength.

EFFECT OF YEAST ON THE DIASTATIC ACTIVITY OF CEREALS

It was shown in a previous experiment (table 8) that mixed unidentified microflora affected variously the diastatic activity of barley, wheat, rye, and wheat flour. The effect of the microflora on the ground wheat and the wheat flour was most pronounced. The stimulating effect of yeast (table 18) on the diastatic activity of flour was also very pronounced.

To study further the comparison between the mixed unidentified microflora and yeast the effect of the latter on the diastatic activity of barley, wheat, and rye was tested. Three grams of yeast were used and no toluene was added to any extractions, as the previous experiment had shown that with this quantity of yeast toluene had little effect. However, toluene was added to the controls.

As indicated in table 19, the yeast stimulated appreciably the diastatic activity of the wheat, depressed to nearly one-half that of barley, and only slightly depressed that of rye. When the effect of the yeast is compared with that of the mixed unidentified flora (table 8) it is seen that the wheat was affected in about the same way, that there was but a slight difference in the effects on rye, but that there was a pronounced difference in the effects on barley; the unidentified micro-

flora caused a slight increase in the diastatic activity of the barley, while the yeast caused a pronounced decrease. It should be noted, however, that while the same varieties of wheat and rye were used in the two parallel tests (tables 8 and 19) the varieties of barley were different. The different response of the two barleys may, therefore, have been due to varietal characteristics.

TABLE 19.—*Effect of yeast on the development of diastatic power in dormant barley, wheat, and rye during different periods of extraction*

Cereal	Variety	Added yeast	Diastatic power for extraction period of—	
			3 hours	24 hours
		Grams	°*L.*	°*L.*
Barley	Tennessee Winter	None	40.1	46.7
		3.0	23.7	22.3
Wheat	Federation	None	55.5	55.5
		3.0	54.1	74.1
Rye	Rosen	None	89.7	97.1
		3.0	74.6	87.6

TOTAL DIASTATIC POWER OF DORMANT CEREAL GRAINS AND FREE DIASTATIC POWER OF MALTS

It was formerly believed that the diastatic activity of barley malts was due to enzymes synthesized during germination in the malting process. The present view is that the amylase is preexistent in the barley grains but that it is liberated or activated during germination (*26*). The liberation of amylase in malt is consequently analogous to the liberation of total amylase in dormant seeds except that in the former it is accomplished by proteinases or activators naturally developed in the seed during germination. The question consequently arose how the total diastatic activity of dormant seeds obtained by means of papain compared with that of malts. Syniewski (*37*) found that barley extracted with a papain solution had a higher saccharifying power than the water extract of the corresponding barley malt. More recently Sallans and Anderson (*33*) have also reported results which show the total diastatic activity of dormant barleys to be much higher than that of the corresponding malts. On the other hand, Hills and Bailey (*19*) and Chrzaszcz and Janicki (*12*) found that the total diastatic activity of dormant barley is lower than the free diastatic activity of the corresponding malts.

In this investigation also the total diastatic power of the cereals studied was compared with that of their corresponding malts. The experimental malts were prepared as follows: 10 gm. of grain, placed in a Petri dish between two layers of filter paper, were wetted with 10 cc. of distilled water, covered, and allowed to stand at 50° F. for 6 days. The germinated cereals were then dried, freed from rootlets and plumules, and ground. The diastatic power of the malts prepared in this way compared well with corresponding malts prepared in the standard way.

It is seen (table 20) that in a general way the total diastatic power of the dormant cereals is correlated with the free diastatic power of the corresponding malts, but that, with the exception of two cases in

which the difference was very small, the free diastatic activity of the malts was appreciably higher than the total diastatic power of the dormant seeds. The results corroborate those of Chrzaszcz and Janicki and Hills and Bailey.

The extent of the increase in diastatic activity of the malts over the total diastatic activity of the dormant seed varies and may be a varietal characteristic.

Hills and Bailey are of the opinion that the increase in saccharifying activity of malts might be due to the alpha-amylase which develops during germination. The striking increase in saccharifying power of the germinated oats may be due entirely to alpha-amylase since the dormant oats are deficient in saccharifying power notwithstanding the fact that they are comparatively rich in proteinases, and since their saccharifying power did not increase when papain was added.

EFFECT OF PAPAIN ON THE DIASTATIC POWER OF MALTS

Hills and Bailey (*19*) came to the conclusion that the beta-amylase content of barley does not increase during the malting process. Papain which increases the saccharifying power of cereals, presumably due to their increase in beta-amylase content, ought accordingly to increase the saccharifying power of malts. Indeed Hills and Bailey found that the addition of papain did increase the saccharifying power of green malts.

In this investigation the total and free saccharifying power of the dry malts obtained from the cereals studied were compared. The experimental malts of the preceding experiment were used in this test.

TABLE 20.—*Total diastatic power in degrees Lintner of different varieties of dormant barley, wheat, rye, and oats compared with the free diastatic power of the corresponding malts*

Cereal	Variety	Free diastatic power in malts	Total diastatic power in dormant seeds	
			Diastatic power	Ratio of dormant seeds to malt
		°*L.*	°*L.*	*Percent*
Barley	Wisconsin Winter	104.7	88.5	84.5
	Tennessee Winter	93.5	79.4	84.8
	Tennessee Beardless No. 6 (C. I. 2746)	65.8	63.7	97.0
Wheat	Federation	126.5	102.0	80.7
	Marquis	160.0	113.7	71.0
	Baart	125.0	106.2	85.2
	Golden	90.9	89.3	90.4
Rye	Dakold	155.0	104.7	67.5
	Star	128.2	103.1	80.6
	Rosen	132.4	109.4	82.7
	Abruzzi	118.4	94.4	79.6
Oats	Fulghum	20.6	3.7	17.9
	Iogold	23.5	2.2	9.4
	Lee	17.7	1.5	8.5
	Winter Turf	19.5	3.0	15.4

It is seen (table 21) that the papain increased the saccharifying power of all malts except those of the oats; in these the papain caused a slight depression in diastatic activity similar to that caused in the dormant oats (table 4). The behavior of the oat malts in this respect

would lend support to the theory that the increase in saccharifying activity of malts caused by papain is due largely to an increase in beta-amylase in which oats seem to be deficient.

TABLE 21.—*Total and free diastatic power in degrees Lintner of malts obtained from different varieties of barley, wheat, rye, and oats*

Cereal	Variety	Diastatic power		
		Total	Free	Ratio of free to total
		°*L.*	°*L.*	*Percent*
Barley	Wisconsin Winter	92.2	83.0	90
	Tennessee Winter	80.6	70.2	87
	Tennessee Beardless No. 6 (C. I. 2746)	58.8	48.1	82
Wheat	Federation	132.4	119.8	90
	Marquis	170.9	138.9	81
	Baart	144.9	104.7	72
	Golden	93.0	77.8	84
Rye	Dakold	137.9	129.0	94
	Star	122.0	118.3	97
	Rosen	127.4	105.8	83
	Abruzzi	104.2	99.0	95
Oats	Fulghum	24.2	25.6	106
	Iogold	22.6	25.6	113
	Lee	16.6	18.1	109
	Winter Turf	16.6	18.1	109

TABLE 22.—*Effect of age on the free diastatic power of malts obtained from different varieties of barley, wheat, rye, and oats (duration of extraction 3 hours)*

Cereal	Variety	Free diastatic power		
		Fresh malt[1]	Malt 8 to 15 months old	Ratio of old to fresh malt
		°*L.*	°*L.*	*Percent*
Barley	Wisconsin Winter	104.7	83.0	79
	Tennessee Winter	93.5	70.2	75
	Tennessee Beardless No. 6 (C. I. 2746)	65.8	48.1	75
Wheat	Federation	126.5	119.8	95
	Marquis	160.0	138.9	87
	Baart	125.0	104.7	84
	Golden	90.9	77.8	86
Rye	Dakold	155.0	129.0	83
	Star	128.2	118.5	92
	Rosen	132.4	105.8	80
	Abruzzi	118.4	99.0	84
Oats	Fulghum	20.6	25.6	124
	Iogold	23.5	25.6	109
	Lee	17.7	18.1	102
	Winter Turf	19.5	18.1	93

[1] Barley and rye malts were prepared in March 1938, wheat malts in September 1938, and oat malts in November 1938.

The increases in saccharifying power caused by papain in the malts of barley, wheat, and rye are consistently lower than those caused by papain in the corresponding dormant seeds (tables 1 to 3). This may be due to the fact that the malts used in these tests were from 8 to 15 months old, and it is shown (table 22) that these malts, again with the exception of the oats, sustained appreciable losses in saccharifying power on aging. These losses may have been sustained largely at the

expense of the beta-amylase content. It was shown above that the saccharifying power of flour extracts, which is presumably due almost exclusively to beta-amylase, also decreases gradually on standing.

The increases in saccharifying power caused by papain in the dormant seeds of the four varieties of barley studied (table 1) compare very well with those reported by Hills and Bailey (*19*) for the green malts of several barley varieties, especially for the 1935 crop. The same authors have shown that their dry malts were lower in saccharifying power than the corresponding green malts. These two facts seem to support the explanation offered above that the discrepancy in this investigation between the respective increases caused by papain in the saccharifying power of the malts and of the corresponding dormant seeds was due to the loss of beta-amylase from the malts during storing.

DISCUSSION

Barley, wheat, and rye show differences in "free" and "total" amylase content between one variety and another in the same species and even more so between one species and another.

The differences between barley, wheat, and rye on the one hand and oats, corn, and rice on the other is particularly striking. It is remarkable that, since the early history of its planned economy, the white race has selected as its staff of life barley, wheat, and rye which have a relative abundance of free and total amylase.

Barley, wheat, and rye differ among themselves not only in their content of total and free amylase but also in the response of their respective diastatic activity to activation by various agents. This was shown particularly by the diverse effect of the acidity of the medium, of the unidentified microflora as well as of yeast, on the diastatic activity of wheat and barley.

While no conclusion is justified from these results regarding the role of variety in the differences of the amylase content, since they represent only 1 year's results, they nevertheless support the already overwhelming evidence that differences in amylase content represent varietal differences. Some observations made in this investigation on differences between varieties of the same cereal with regard to their amylase activity, if sustained, suggest the possibility that varietal differences which could not be detected by ordinary analytical methods are to be looked for in this biological field. Varieties may differ not only with respect to total and free amylase but also with respect to the quantitative relation of the two kinds of amylase (alpha and beta), to the way in which their diastatic activity is stimulated, to their proteinase activity as indicated by their effect on a medium such as flour which is rich in amylase but poor in active proteinase, and with respect to the optimum pH digestion of their proteinases. These differences may be responsible for the differences in the baking quality of the flour produced from different varieties of wheat. Differences in the optima of digestion of the proteinases may perhaps account for the fact that one variety of a cereal is sometimes more affected by the acidity of a soil than another variety of the same cereal.

In the group of the sorghums and noncereal seeds there is a striking difference between soybeans, which have a relative abundance of amylase, and the other seeds which have little or no amylase. The

presence of a relatively large quantity of amylase in soybeans is somewhat surprising since they are practically devoid of starch. It would seem, therefore, that amylase has some other function in plant metabolism besides the transformation of starch into sugar.

The results obtained in this investigation also throw some light on the mechanism of stimulation of amylase activity in seeds. The analogy between the theory that the so-called bread improvers inhibit the excessive proteolysis of the flour proteins by natural proteinases found in flour and the theory that amylase in cereals is combined with proteins and is set free by proteinases naturally found in the cereals or added proteinases, is clearly brought out. These two theories lend mutual support to each other. Added proteinases like papain, pepsin, etc., which decrease the loaf volume of bread also liberate amylase in flour. Chemical oxidants which increase the loaf volume of bread, presumably by inhibiting the flour proteinases, also depress the diastatic activity of the flour.

The analogy between the two theories is also strengthened by the identical arguments made against them by their respective opponents. Chrzaszcz and Janicki, who oppose the theory of amylase liberation of Myrbäck, and Read and Haas, who oppose the theory of Jørgensen, claim respectively that the increase diastatic activity caused by proteinases is not commensurate with the increase in nitrogen solubility in the case of the former theory and that the depression in nitrogen solubility is not commensurate with the increase in the loaf volume of bread in case of the latter theory. The answer to the respective arguments, as was pointed out in the discussion of the corresponding individual experiments, is also identical: not all the proteins are involved in the analogous phenomena covered by the two theories but only a portion of them. When the increase in diastatic activity caused by proteinases, such as papain, is correlated with their effect in decreasing the loaf volume of bread or when the increase in loaf volume of bread caused by bread improvers such as potassium iodate is correlated with their effect in depressing diastatic activity there is a direct relation between the concentration of the causal agents and their effect in the two sets of phenomena.

While there is a distinct correlation between decrease of loaf volume and stimulation of diastatic activity caused respectively by proteinases, such as papain, and by bread improvers, such as potassium iodate, there is evidence which tends to show that the changes in amylase activity referred to are not directly responsible for the corresponding changes in loaf volume. The amylase changes serve only as indicators of some other changes, probably of changes in the proteins due to the activity of the proteinases which liberate the amylase.

It was shown that several of the seeds studied are poor in amylase but possess appreciable proteinase activity, which was demonstrated by their effect in stimulating the diastatic activity of flour. This suggests a possible new method for the determinztion of the gross proteolytic activity which might, in certain instances, take the place of the more laborious nitrogen-solubility, viscosity, or titration methods. Similarly, the quantitative relation between free and total amylase of a biological material may also serve under certain conditions as an indication of the proteinase strength of these materials,

especially of proteinases which are factors in determining the baking qualities of flour.

While most of the facts and observations brought out in this investigation favor the proteolytic mechanisms of amylase liberation suggested by Myrbäck and others, the effect of the nonproteinase agents on the liberation of amylase could be interpreted as lending some support to the theory of sisto-substances and eleuto-substances of Chrzaszcz and Janicki. Thus cysteine and sodium chloride could be considered as eleuto-substances while the depressing effect of aging on the amylase activity of flour could be attributed to a sisto-substance. The character of activity of the nonproteinase agents varies. Cysteine, for instance, like the proteinases, stimulates diastatic activity and depresses the loaf volume of bread; the effect of aging is similar to that of a bread improver; it depresses diastatic activity and increases the loaf volume of bread. On the other hand, sodium chloride and micro-organisms stimulate diastatic activity without decreasing the loaf volume of bread to the same extent as the proteinases. There is still therefore the question whether the nonprotein agents act directly on the amylase and proteins or on the proteinases. Balls and Hale came to the conclusion that cysteine acts on the proteins and that the effect of aging is connected with proteinase activity. It is possible that the stimulating effect of peptone on diastatic activity which is Chrzaszcz and Janicki's strongest proof in favor of the existence of eleuto-substances, is in reality due to its effect on microbiological activity.

SUMMARY

Dormant seeds of barley, wheat, rye, oats, corn, rice, buckwheat, soybeans, sorghums, and cowpeas and also ordinary white flour were analyzed for total and free amylase.

Of the principal cereals, wheat showed the highest diastatic activity; rye was close to wheat and barley was considerably below wheat; oats, corn, and rice showed very little diastatic activity.

Of the other seeds studied soybeans showed a relatively high diastatic power; the buckwheat, cowpeas, and sorghums had a very low saccharifying power or none.

Each of the four varieties of barley, wheat, and rye showed a variation in the amylase content. This variation was consistent for the total as well as for the free amylase.

The diastatic activity developed in barley, wheat, and rye with the addition of papain (total amylase) was invariably higher than that developed without the addition of papain (free amylase). Papain had no effect on the saccharifying power of soybeans nor on that of the seeds which had a low free amylase content.

Ordinary white wheat flour showed a much lower free diastatic power and a higher total diastatic power than ground whole wheat.

Baker's yeast and certain common unidentified microflora increased the diastatic power of ground whole wheat and flour but had little effect on rye and oats; baker's yeast decreased the diastatic power of barley but the microflora had little effect on it.

Lactic acid increased the diastatic power of flour and ground wheat and depressed that of barley. There is a possibility that the effect of lactic acid is due to a shift in the pH of the respective extracts. In

the case of wheat this shift is toward the optimum digestion of the wheat proteins; in the case of barley it is away from that optimum.

Sodium chloride increased the diastatic power of wheat and flour and decreased that of barley and rye. In flour the liberation of amylase was related to the concentration of the salt.

There is evidence that the effect of lactic acid, microbiological activity, and sodium chloride on the diastatic activity of the cereals varies with the variety of the cereal.

There is evidence supporting the theory on the mechanism of liberation of amylase in cereals advanced by Myrbäck and others; namely, that the amylase is combined with proteins and is liberated by proteinases naturally found in cereals or by added proteinases.

A clear analogy is established between the theory with reference to the mechanism of the liberation of amylase in cereals by proteinase activity and the theory with reference to mechanism of action of the bread improvers; namely, that the chemical oxidants inhibit the degradation of the flour proteins by proteinases naturally found in it. There is evidence that the same kind of proteins and proteinases are involved in the two analogous phenomena. The two theories mutually support each other.

The decrease of the loaf volume of bread and the liberation of amylase in flour were respectively correlated with the same concentrations of papain.

Potassium iodate, which is used as a bread improver, depressed the diastatic power of flour; the depression was correlated with the concentration of the iodate.

Cysteine, like sodium chloride, stimulated the diastatic activity in flour, the stimulation being related to the concentration of the stimulant. However, cysteine decreases the loaf volume of bread, while sodium chloride does not, which shows that there is some difference in the mode of action between these two nonproteinase stimulants of diastatic activity.

Flour was found to be rich in amylase and poor in active proteinases. It was also found to be a good medium for testing the effect of proteinases and for the estimation of proteinases in other plant materials.

By using flour as the testing material it was found that the proteinase content in plant materials was not correlated with their amylase content. Barley and rye with a relatively high amylase content showed the lowest proteinase content of all the seeds studied. Of the principal cereals, oats, which have a relatively low amylase content, had the highest proteinase content. Of all seeds studied soybeans, with a high amylase content, had also the highest proteinase content. The next highest proteinase content was that of cowpeas, which have a low amylase content. Wheat germ and bran have a lower total amylase content and higher proteinase content than flour. The germ had a higher proteinase content than the bran.

The free diastatic power of the malts prepared from cereals was higher than the total diastatic power of the same dormant cereals. Papain increased the diastatic power of these malts.

The experimental malts prepared from barley, wheat, and rye lost part of their diastatic strength with aging.

Apparently the increase in free diastatic power of malts over that of the corresponding cereals is due largely to an increase in alpha-amylase.

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PATHOLOGICAL HISTOLOGY OF SUGARCANE AFFECTED WITH CHLOROTIC STREAK [1]

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INTRODUCTION

Chlorotic streak is one of the least understood diseases of sugarcane. Potentially it is of considerable economic importance, since it is known to cause a marked reduction in the germination of plant cane and ratoons; this effect, together with the later stunting of growth, results in severe losses in tonnage. Such losses have occurred in localized areas of Louisiana, where infection reached a fairly high level before control measures were instituted. The history of the discovery and relatively rapid spread of the disease in that State conforms to the pattern for virus diseases, and the proof that it is transmitted by the leafhopper *Draeculacephala portola* (Ball) [2] not only throws light on the spread of the disease but also gives strong indication that it is caused by a virus. However, in earlier work Carpenter [3] interpreted certain bodies found in association with chlorotic streak as fungi. In the present study of the pathological histology of the disease an opportunity was afforded to search again for organisms that might be associated with it.

The most reliable diagnostic symptom of the disease is the appearance on the leaves of chlorotic streaks, which vary in length from a few millimeters to nearly the entire length of the leaf. The streaks are distinguished from other streaks occurring on sugarcane leaves by their broken, wavy, irregular margins. Abnormal stiffness and straightness of the foliage, especially of young plants, and reddening of vascular bundles in the stalks, particularly in the nodal region, frequently occur in diseased plants. However, these symptoms have not been sufficiently differentiated from similar effects produced by other causes to be relied upon for positive identification of chlorotic streak in the absence of the characteristic streaks.

Chlorotic streak apparently is not a completely systemic disease. All aerial parts of the plant may be affected to some degree at one time or another, but there is evidence that many plants are not completely invaded, and, even after the disease has developed, apparently complete recovery may occur.[4] In a disease of such erratic behavior, it is particularly desirable to ascertain the sequence in its histological development with respect to the tissues affected. This study of the pathological histology of sugarcane affected with the disease was undertaken as an aid to a better understanding of its nature and its effects on the plant.

[1] Received for publication June 17, 1943.

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[4] Unpublished data of the senior author.

MATERIALS AND METHODS

The C. P. 29/320 variety of sugarcane was used in most of this work. Material was taken from the dormant and sprouting axillary buds, young shoots, old stems, and leaves of diseased and healthy plants.

The principal killing fluids used were formalin-acetic-alcohol (F. A. A.), Bouin, and various modifications of Nawashin's formula (Craf). Although all of these yielded generally satisfactory sections, the Craf formulas gave the best preservation of cells. For basic fixation the Erlicki-Zirkle formula was used.

The dehydrants and solvents used in various combinations were acetone, ethyl alcohol, ethyl ether, chloroform, tertiary butyl alcohol, anhydrous dioxane, and xylol. Progressive infiltration in Parowax was followed by casting in commercial Tissuemat or in a similar matrix compounded with gum rubber, beeswax, and paraffin. The hot-celloidin and cold-celloidin methods were also employed.

Of the various stains and combinations used, iron-alum haematoxylin and safranine and fast green were preferred. The specific methods used are indicated in the text and in some of the explanations of plates.

HISTOLOGICAL STUDIES

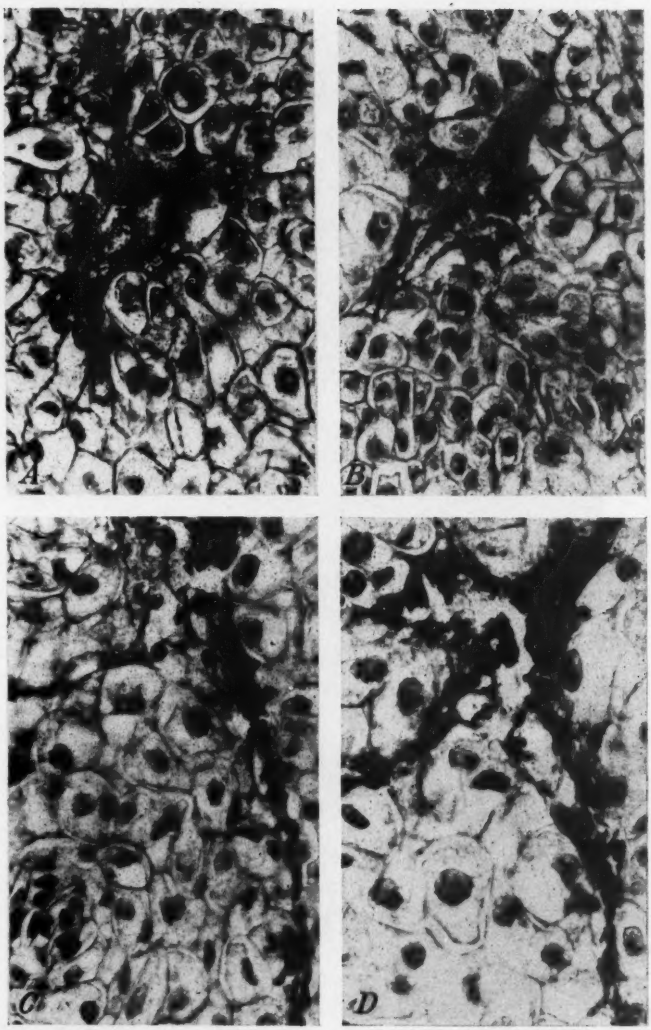
BUD

Axillary buds were taken at random from mature, field-grown, diseased stalks and embedded as just described. Sections from most of these showed no histological abnormalities when compared with healthy material. Some of these buds may not have been invaded by the disease, since it is known that buds on diseased stalks may give rise to apparently healthy plants. However, from the large number of buds studied and their random selection from many stalks, it may be assumed that at least some of them were invaded and would have produced diseased plants.

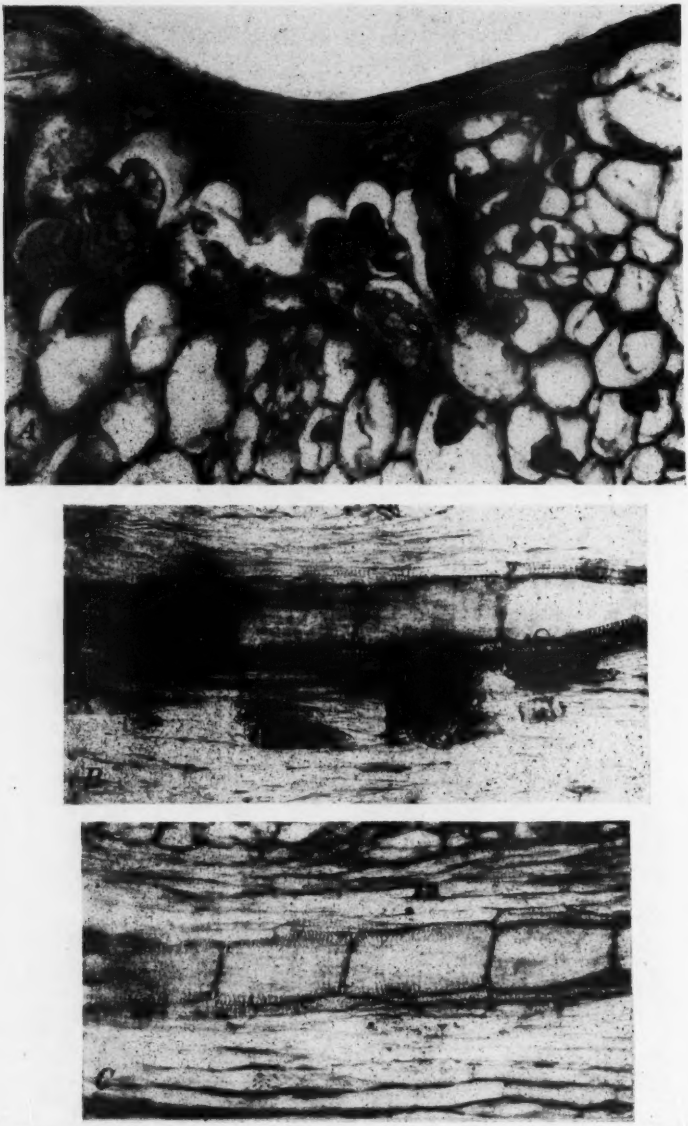
In some buds there were marked pathological disturbances. Several or all of the differentiating leaves showed necrosis in varying degrees, and in some leaves large necrotic cavities had developed (pl. 1, *A*, *B*). Necrosis was accompanied by the abundant production of a dark-staining gum. The gum filled the cavities as an amorphous mass or was in the form of spherical globules of varying size, some of which assumed a pelliclelike covering resembling a wall. The presence of these spheres in a necrotic strand is shown in plate 1, *C*, *D*. Some are teardrop-shaped; others are rounded and have a wall-like covering.

Frequently the necrosis was initiated in the epidermis of the adaxial surface of the differentiating leaves, and sometimes it extended for some distance along the epidermal layer with only slight penetration into the mesophyll (pl. 2, *A*). Often, however, the mesophyll was involved along with the epidermis, and in some leaves the necrotic condition was limited to the parenchyma.

It is improbable that a bud showing as severe necrosis as that illustrated in plate 2 would germinate, and since one of the effects of chlorotic streak is the suppression of germination of the buds on planted cuttings and ratoons, it may be assumed that the failure to germinate is at least partly the result of the necrotic condition described. On several occasions, buds from diseased stalks that had



Necrosis in diseased axillary buds. *A, B,* Necrotic cavities developing in the mesophyll. ×400. *C, D,* Necrotic strands radiating from cavities in the leaf parenchyma of ungerminated buds, with spherical globules appearing in the mass of gum. ×400.



A, Necrotic area in a diseased bud, initiated in the epidermis and extending into parenchyma; arrow indicates plasmodiumlike extrusion from one cell into another. ×800. *B*, Unstained fresh section of stem tissue prior to dehydration, showing clumps of a translucent, granular substance resembling plasmodiumlike bodies. ×800. *C*, The section shown in *B*, after dehydration in dioxane; note the almost complete dissolution of the substance. ×800. Similar dissolution occurred in several fat solvents, particularly in xylol.

failed to germinate after several days' incubation at 35° to 36° C., which is optimum for germination of sugarcane, were found, on sectioning, to have necrotic areas.

LEAF

A histological study was made of the progressive development of the leaf streaks from their incipiency to necrosis. Stained sections of embedded material were used for the study. The streaks may make their first appearance on a leaf at any stage of development, from the time it unfolds from the spindle until senescence. At first the streaks are very faint chlorotic lines with irregular margins, but they may increase rapidly in size until they extend most of the length of the leaf and may broaden to approximately half an inch in width. Within as few as 7 days the centers of the streaks may become necrotic. Frequently, however, the streaks enlarge very little from their original size, remaining small and faint throughout the life of the leaf; and many of the streaks that do increase in size do not become necrotic. The streaks retain the characteristic irregular margins, regardless of the size they attain.

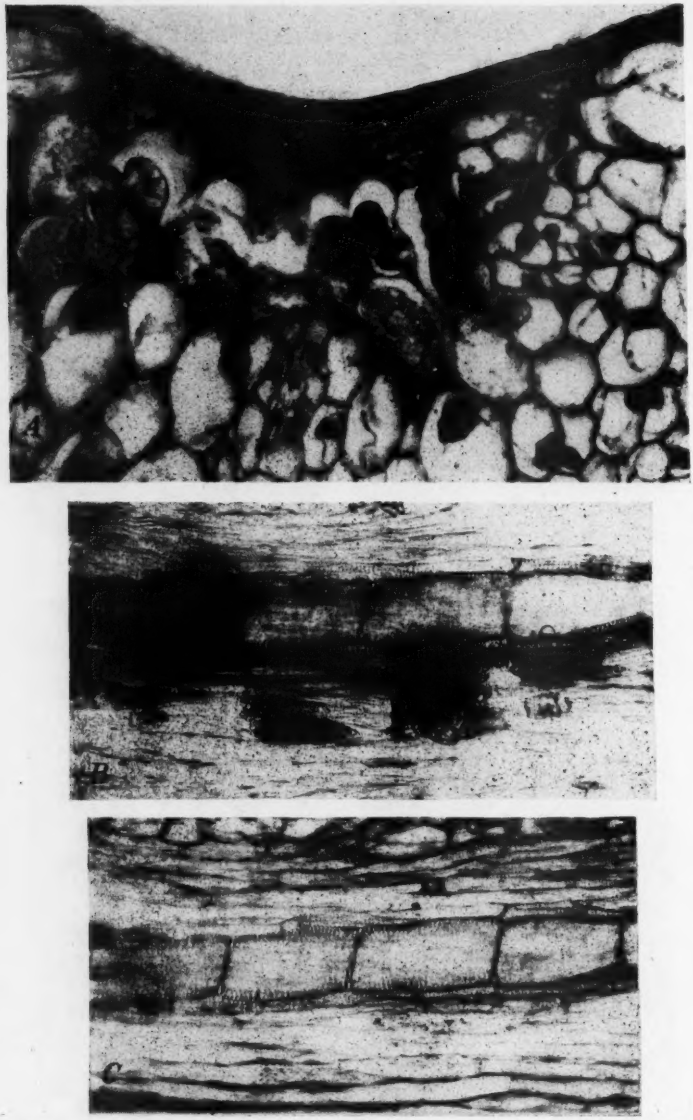
Leaves of germinating buds and of young shoots were studied to determine whether incipient streaks could be detected prior to the unfolding of the leaf. In the material studied, plastid development appeared to be normal and no marked tissue abnormalities were recognized at this stage.

Some leaves on diseased plants, particularly the first leaves produced, do not develop well-defined streaks but have an over-all "scalded" appearance, with poorly defined chlorotic areas. Frequently the tips and margins of the leaves become dried. In the field, this is particularly noticeable during prolonged dry weather in the spring, when the plants in areas of high infection may have a scorched appearance.

In these "scalded" leaves, as in the prominently streaked areas of other leaves, the chloroplasts are markedly reduced in number, size, and stainability. In some cells of the mesophyll the protoplast may be clumped into a dark-staining mass. Thickening of the walls of mesophyll and epidermal cells, a process that also precedes necrosis in the streaked areas, may occur. Similar histological disturbances may develop in the bundles. Walls of either phloem or xylem, or both, may be abnormally thickened, and both may show various amounts of a gumlike deposit. It is only in the "scalded" leaves that an abnormal condition of the phloem has been observed in advance of necrosis in the mesophyll.

The streaks may appear on only one or on several leaves of a diseased plant. In Louisiana it is comparatively rare for all the leaves of a plant to exhibit the symptoms at one time, and there may be periods in the life of a diseased plant during which it is symptomless so far as the leaf streaks are concerned.

Chloroplasts in the incipient streaks show a distinct reduction in number and size, a loss of stainability, and pronounced clumping as compared with chloroplasts in the adjacent tissues. Frequently the thickness of the streaked portion of the leaf is also reduced, and there is a tendency toward excessive brittleness of these areas when micro-



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Some leaves on diseased plants, particularly the first leaves produced, do not develop well-defined streaks but have an over-all "scalded" appearance, with poorly defined chlorotic areas. Frequently the tips and margins of the leaves become dried. In the field, this is particularly noticeable during prolonged dry weather in the spring, when the plants in areas of high infection may have a scorched appearance.

In these "scalded" leaves, as in the prominently streaked areas of other leaves, the chloroplasts are markedly reduced in number, size, and stainability. In some cells of the mesophyll the protoplast may be clumped into a dark-staining mass. Thickening of the walls of mesophyll and epidermal cells, a process that also precedes necrosis in the streaked areas, may occur. Similar histological disturbances may develop in the bundles. Walls of either phloem or xylem, or both, may be abnormally thickened, and both may show various amounts of a gumlike deposit. It is only in the "scalded" leaves that an abnormal condition of the phloem has been observed in advance of necrosis in the mesophyll.

The streaks may appear on only one or on several leaves of a diseased plant. In Louisiana it is comparatively rare for all the leaves of a plant to exhibit the symptoms at one time, and there may be periods in the life of a diseased plant during which it is symptomless so far as the leaf streaks are concerned.

Chloroplasts in the incipient streaks show a distinct reduction in number and size, a loss of stainability, and pronounced clumping as compared with chloroplasts in the adjacent tissues. Frequently the thickness of the streaked portion of the leaf is also reduced, and there is a tendency toward excessive brittleness of these areas when micro-

tomed. As the streaks increase in size, there is an extension of the area with reduced chloroplasts.

Necrosis begins with the disorganization of the protoplast, culminates as a rule in the darkening and dissolution of the cell wall, and is accompanied by a heavy deposition of gum. In fixed material the entire cell contents may be clumped into a dark-staining mass (pl. 3, *E*). Usually the mesophyll or the border parenchyma is affected in advance of the vascular tissue, although in some instances bundles showing marked necrosis are surrounded by apparently normal tissue, except for the reduction in the number and size of chloroplasts.

As necrosis proceeds, the cell walls become wrinkled in outline and presently collapse, but as a rule they do not rupture until necrosis is far advanced. Splitting of the leaf along the necrotic streak may occur, although the streak may remain intact during the life of the leaf.

In the affected cells of necrotic leaf tissue there is usually profuse gummosis. The gum is apparently of the same nature as that occurring in the stems. Some of the cell inclusions consist of spherical globules of varying size, some of which may develop a pellicle-like outer layer resembling a wall (pl. 3, *E*, *F*). Some of these bodies occur in apparently normal tissues of the diseased leaf. Regardless of size and of their apparent resemblance to definite organized bodies, however, the contents of these bodies have been found to be uniformly homogeneous, with no evidence of nuclear structure.

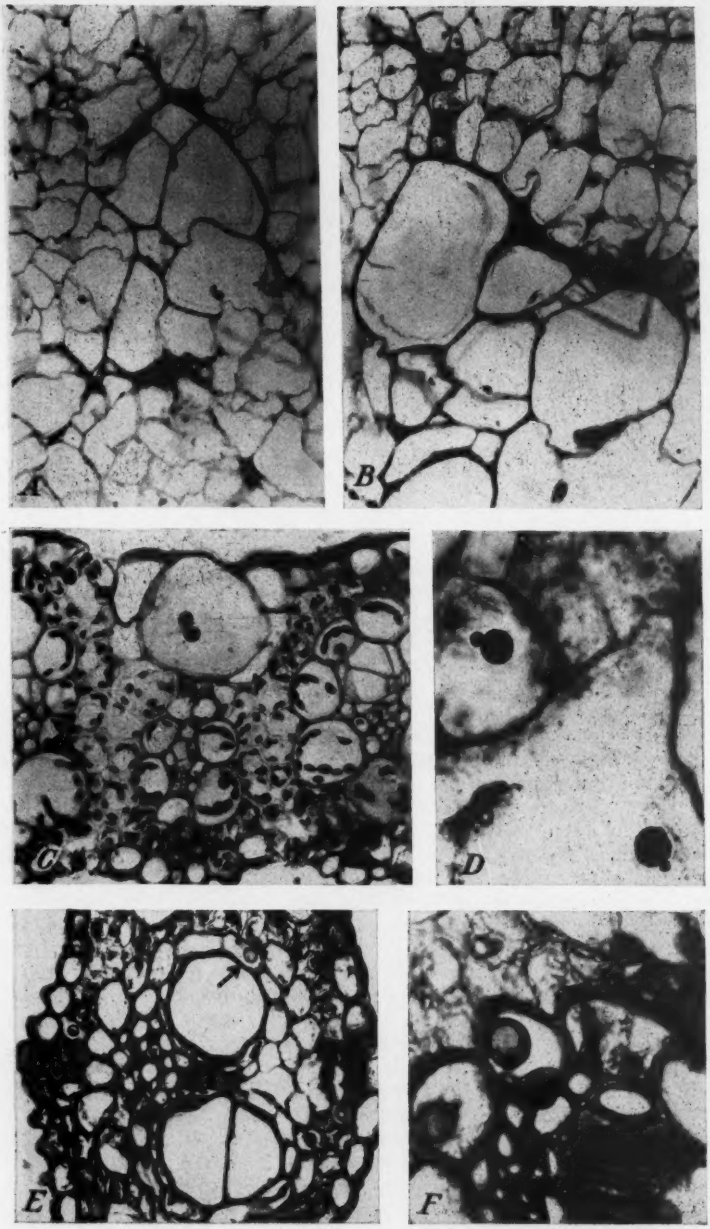
Other intracellular inclusions, the origin of which is less obvious, occur in the diseased leaves. Two of these in a bulliform cell are shown in plate 3, *C*.

STEM

Several hundred single-bud cuttings were made from both diseased and healthy stalks; the latter were treated with hot water at 52° C. for 20 minutes to insure freedom from the disease, and were germinated in steamed soil in the greenhouse. Material for embedding was taken at the time of emergence of the shoot from the soil, when the shoots were 6 to 8 inches tall from the dewlap, and again when they were 12 to 18 inches tall. At this last stage no histologically mature internodes had emerged above soil level.

Tissues of the growing point and young internodes of diseased shoots appeared to be normal. Evidence of the presence of the disease was not found until internodes of the shoot approached histological maturity. In these internodes necrotic strands in the pith parenchyma were observed, in some instances involving only a few cells, but in others extending for some distance through the parenchyma. Necrosis was also found sporadically in the pith parenchyma of mature field-grown stalks (pl. 3, *A*, *B*), in which a partial or complete dissolution of the cell wall occurred. In regions having extensive necrosis of parenchyma, the bundles remained as islands of apparently normal vascular tissue enclosed in necrotic parenchyma. This is similar to the pattern of necrosis observed by Sartoris [5] in stem necrosis of nonparasitic origin and suggests that the development of necrosis may follow the same general course regardless of cause. Un-

[5] SARTORIS, G. B. NECROTIC STRIPES IN SUGARCANE. Jour. Hered. 31: 515–520, illus. 1940.



A, B, Necrosis of parenchyma of mature, field-grown stalks. ×900. *C,* Spherical bodies in a bulliform cell of a diseased leaf at some distance from a streak. ×320. *D,* Dark-staining, spherical bodies in the nodal region of young diseased shoots. ×900. *E,* Necrosis in leaf tissue, showing collapse of the mesophyll cells surrounding a large bundle; the sheath cells are also collapsing, and deposit of gum is shown in the conductive elements; arrow indicates a spherical, "walled" body. ×400. *F,* Enlargement of a necrotic area similar to that in *E,* showing a spherical body. ×800.

like the condition found in the leaf, general necrosis of stem bundles was not observed.

Necrosis is associated with the production of abundant deposits of materials of undetermined character, which, for convenience, will be termed "gummy substances." These gums may occur as intercellular masses or strands of homogeneous materials, yellowish or reddish in the unstained condition and stainable with safranine. Necrotic cavities of intercellular origin, which eventually include a zone of collapsed parenchyma, are filled with the gum, occurring either as irregular masses and strands (pl. 3, *A*, *B*) or as discrete globules. The gum may be completely amorphous or it may exhibit some striation and minute granularity. Occlusion of xylem elements often occurs, and less frequently of the phloem also. In view of the varying degrees of gum organization, from amorphous masses to sharply defined globules, the gummosis shown in plates 1, 2, *A*, and 3, *A*, *B*, may well be of essentially the same character.

A further advance in the apparent organization of secretions is exhibited by intracellular spherical bodies of the same nature as those observed in the leaves (pl. 3, *D*). In size they range from minute dots to large spheres that nearly fill the cell. They resemble the gum globules just described but have a more pronounced pelliclelike covering. Many of the bodies have protuberances, which, in fresh material, bear a superficial resemblance to budding or germination. Bodies of essentially identical structure are known to occur in leaves of various hosts infected with several species of rust. Although they are found most frequently in the nodal region, their distribution is not limited to any particular portion or tissue of the stalk. One or several may occur in a cell and may lie close to the nucleus without any evident effect on it. Rarely the nucleus partially surrounds the body, although not coming in direct contact with it, but this may be a mere physical accommodation to the presence of a foreign substance. The inclusions stain readily with safranine, iron-alum haematoxylin, and gentian violet, but are not stained by the Feulgen technique. In spite of their reaction to most nuclear stains, however, their contents are uniformly homogeneous, with no indication of a nuclear structure. Dufrenoy's description of coacervates [6] suggests these bodies, except that in the present study they have not been found in plants known to be free of chlorotic streak.

One phase of the study reported here was concerned with a search for a tangible causal organism; therefore, no intensive effort was made to demonstrate the cellular inclusions and abnormalities known to be associated with some virus diseases. Such studies are now in progress. The only abnormality to be reported at present is the presence of marked attenuations and lobes on many of the nuclei in diseased tissues. Although the nuclei of healthy tissues exhibit some small, pointed processes, the incidence of lobing, forking, and bizarre-shaped nuclei is much greater in diseased plants.

In the lower internodes of many diseased stalks, and particularly in those below the soil level, there are sporadic accumulations of clumps or strands of a gelatinous substance, which, in the fresh material, suggests organized plasmodiumlike bodies. Some of these

[6] DUFRENOY, J. VACUOLAR INCLUSIONS IN CELLS OF SUGAR CANE AFFECTED WITH CHLOROTIC STREAK. (Abstract) Phytopathology 32: 3. 1942.

are spherical, some are elongate and sinuous, and some are highly irregular in outline. In size they vary from small spheres to clumps of globules that appear to fill some cells. In its more granular texture and by the presence of many small, dark, highly refractive particles, this substance differs from the amorphous masses and discrete spherical globules of gum described previously. Some of the clumps are translucent; others are dense, dark-colored, and opaque. They occur in the pith parenchyma and in vascular tissues of the stems, more abundantly in young shoots than in old stalks, but they have not been observed in the leaves; nor have they been found in plants known to be free of chlorotic streak. These bodies appear to be similar to those interpreted by Carpenter [7] as chytrids.

Numerous attempts were made to preserve these bodies by the paraffin and celloidin methods. Freehand sections were killed and examined as wet mounts in the killing fluid. The plasmodiumlike bodies were located, and the dehydrating agent was introduced under one side of the cover glass and drawn through with blotting paper. The graded dehydrating series customarily employed in the paraffin process was used. The bodies were thus under observation during the entire process of dehydration and transfer to the paraffin solvent. By selecting fields of view having distinctive features, such as the series of trachea in plate 2, *B*, *C*, it was possible to locate and photograph the same field at any stage of the process, even after the slide was subjected to an interval in the oven.

In these experiments, some shrinkage of the plasmodiumlike material occurred in formalin-acetic-alcohol, but the substance remained intact through prolonged storage in these fluids. It persisted during dehydration with ethyl alcohol, tertiary butyl alcohol, and dioxane, but disappeared almost completely in xylol, chloroform, and ethyl ether; and in anhydrous dioxane at 53° C. In some instances, fragments of the material persisted until paraffin infiltration was begun, but they disappeared completely during infiltration at 53°. The slow process of embedding in cold celloidin was also tried, and in some instances the substance was preserved. However, in the sections cut from this embedded material, the depositions disappeared when the stained slides were cleared in xylol.

The techniques used in this study preserve with satisfactory fidelity the plasmodia and nuclei of *Synchytrium*, *Plasmodiophora*, and some other Myxomycetes. It is improbable that all stages of the life history of a thalloid pathogen in chlorotic streak could have eluded the present investigators.

When fresh or preserved sections were stained with safranine and fast green, the safranine was not retained by the bodies described above; the green dye was taken up to about the same extent as by the host cytoplasm, and a metallic sheen was imparted to the dark particles within the masses. There was no evidence of nuclear structure in the masses in either freehand or fixed material. In freehand sections stained with Sudan III, the masses took on the brilliant color of the dye. This, together with its solubility in fat solvents, suggests that the material may be fatty. While this material has not been definitely identified, it is apparent from the evidence presented that it is nonliving, and it is believed to be a product of the metabolism of diseased plants rather than a causal agent.

[7] See footnote 3, p. 201.

SUMMARY

A study was made of the pathological histology of buds, leaves, and stems of sugarcane affected with chlorotic streak. Some axillary buds from diseased stalks showed no histological abnormalities; others exhibited varying degrees of necrosis of epidermis and mesophyll.

Development of the characteristic streaks on the leaves resulted in marked reductions in the number, size, and stainability of the chloroplasts in the mesophyll and chlorophyll-bearing bundle-sheath cells. When necrosis developed in these streaks, it was initiated in the mesophyll and involved the vascular bundles only after destruction of mesophyll tissue was far advanced. In diseased leaves having a "scalded" appearance, but lacking the distinctive streaks, thickening of the walls and gummosis sometimes occurred in the xylem and phloem in advance of, or simultaneously with, similar degeneration in the mesophyll.

Necrosis of parenchyma tissue of stems was found sporadically. Gummosis of the conductive elements was common, but no other abnormalities were observed in these tissues. The gumlike material may be completely amorphous; it may exhibit some striation and minute granularity; or it may be aggregated into spherical or teardrop-shaped globules.

Spherical intracellular bodies that stained deeply with safranine and iron-alum haematoxylin were found in stems and leaves of diseased plants. Although some of these had a pelliclelike covering resembling a wall, their contents were homogeneous, with no indication of nuclear structure.

Accumulations of a gelatinous substance, frequently resembling organized bodies, were observed in the stems of some diseased plants. The material was soluble in ether, xylol, dioxane, and chloroform, but not in ethyl alcohol, and stained brilliantly with Sudan III, indicating a fatty chemical character.

The various types of gummosis and the bodies exhibiting some degree of structural organization are believed to be products of the metabolism of diseased plants, rather than causal agents.

THE Ms_2 A V_{10} LINKAGE GROUP IN SORGHUM[1]

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INTRODUCTION

The male-sterile character in sorghum (*Sorghum vulgare* Pers.) offers the most feasible method yet devised of producing first-generation hybrid seed in large quantities and of thus utilizing the hybrid vigor found in certain sorghum crosses for obtaining high yields. Since male-sterile plants obviously are not self-propagating and the character must be maintained in a heterozygous condition, all pollen-producing segregates must be removed from the seed-parent variety growing in a crossing block. Such plants must be destroyed before any pollen is shed, and the earlier they can be removed from competition with the ultimate seed-producing plants the greater will be the acre yield of hybrid seed. Any easily recognized plant character that is closely associated with male sterility by genetic linkage would permit the removal of most of the pollen-producing plants before the pollination stage is reached. Data are presented to show the association of factor pairs for awnless and awned lemmas and for green and virescent-yellow plants with the male-sterile factor pair, Ms_2ms_2.

REVIEW OF LITERATURE

Male sterility in sorghum was reported by Rangaswami Ayyangar and Ponnaiya (*1*)[2] and by Stephens (*15*), in 1937. In each case anthers were devoid of pollen but stigmas were receptive; monogenic inheritance was observed with male sterility recessive; and the symbols *Msms* were used for the factor pair involved. Karper and his associates (*9*) reported two additional male steriles and designated them ms_3 and ms_4, but they gave no data regarding inheritance.

In the cross Dwarf milo × feterita, Vinall and Cron (*18*) reported the F_1 unawned and a segregation of 3 awnless to 1 awned in the F_2. Sieglinger et al. (*14*) described milo as strong-awned, feterita as tip-awned, and the F_1 as weak-awned and found that the segregration was 1 strong-awned, 2 weak-awned, and 1 tip-awned in the F_2 of crosses between strong-awned and tip-awned varieties. They considered strong-awned partly dominant to tip-awned. In crosses involving awnless and either tip-awned or strong-awned they found the awnless condition to be almost completely dominant in the F_1. They reported 3 : 1 segregations in the F_2 with awnless dominant.

[1] Received for publication June 26, 1943. Cooperative investigations of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, and the Texas Agricultural Experiment Station at Substation No. 12, Chillicothe, Tex. Technical Series No. 777, Texas Agricultural Experiment Station. Field and office assistance was furnished by personnel of the Works Progress Administration, Official Project No. 265-1-66-60.

[2] Italic numbers in parentheses refer to Literature Cited, p. 217.

To explain their results they suggested multiple allelomorphs and used the symbols *AA* (awnless), *aa* (strong-awned), and a^ta^t (tip-awned) for the factors involved. They also recognized homozygous weak-awned strains but did not report on the inheritance of this type of awn development. Ramanathan (*12*) reported short awns dominant to long awns, and Karper (*6*) and Sieglinger (*13*) reported awnless dominant to awned.

Rangaswami Ayyangar and Reddy (*2*) reported a type of stigma on which the brushy or feathery branches occupied only the lower third of the axis while the upper two-thirds was devoid of branches. They found basal-feathered stigmas to be recessive to normal, or full-feathered, with monogenic inheritance (*3*). They also reported that in most groups of sorghum leaf tips are hairy but that in a few groups they are usually glabrous (*4*). Hairy leaf tip was a monogenic dominant to glabrous leaf tip. In crosses where both pairs of characters were brought together in the coupling phase, these workers found linkage with 25 percent crossing over. The factors for each of the characters were also linked with those for awns. In the F_2 and F_3 populations crosses with leaf tip hairy vs. glabrous and awns absent vs. present gave 43 percent crossing over in the repulsion phase, and crosses with awns absent vs. present and stigmas full-feathered vs. basal-feathered gave 18 percent crossing over. These data established the existence of a linkage group that includes the factor for the presence of awns, with percentages of crossing over between successive factor pairs in the following order: Awns (18) stigma feathering (25) leaf tip hairiness. Each pair of this group was reported to be independent of factors for plant color (*Pp*), wholeness of grain color (*Ww*), and hairiness of nodal band. Sieglinger (*13*) reported awns independent of seed-color factors in the F_2 of a Blackhull kafir × darso cross, and Stephens and Quinby found no association of awns with members of the *Q B Gs* (*16*) and *D Rs P* (*17*) linkage groups.

A number of chlorophyll-deficient types, ranging from those with weak zygotes, some of which fail to emerge, and from albinos lethal as soon as endosperm food reserves are exhausted, to pale greens that can hardly be separated from normal siblings, have been reported in sorghum. Karper and Conner (*8*) designated two virescents found in Blackhull kafir v_1 and v_2 and showed data indicating monogenic inheritance in each case. A third chlorophyll deficiency, described in the text as pale yellow but in the summary as virescent, was assumed to be a simple recessive. This virescent was later (*6*) designated v_3 and reported independent of seedling red stem (*Rs*), w_1 and w_2 (albinos), and y_2 (lethal-yellow seedling). In 1935, Karper (*7*) reported six virescents obtained from X-rayed seed, and these are the six for which Martin (*11*) used the symbols v_3 to v_8, inclusive, with the data credited to Quinby and Karper. The symbols for the X-ray virescents should have been reported to Martin as v_4 to v_9, inclusive. It has not been determined definitely that no duplications occur within this series.

DESCRIPTION OF CHARACTERS

MALE STERILITY

In typical expression, anthers of male-sterile (ms_2ms_2) plants are one-half or less the size of normal anthers and do not contain pollen (fig. 1, *A* and *B*). Though readily located in normal siblings, no pollen mother cells could be found in male-sterile plants, indicating that

FIGURE 1.—Flowers of normal (*A*) and male-sterile (*B*) Texas Blackhull kafir, showing relative size of anthers. Panicles of normal (*C*) and male-sterile (*D*) Texas Blackhull kafir. Note pointed apex of *D*.

action of the gene takes place before the formation of pollen mother cells. However, in occasional populations the anthers do reach a more advanced stage, and in at least three of the populations grown, scattered normal flowers have been observed on male-sterile panicles. In the Texas Blackhull kafir stock, male-sterile panicles are distinctly more pointed than normal panicles, so that one can make fairly accurate separations of phenotypes by observing panicle shape as heads are exserted from the upper sheaths (fig. 1, *C* and *D*). Ordinarily the panicle of Blackhull kafir grown under favorable conditions has a rachis 60 to 80 percent of the total panicle length, terminated by a cluster of branches (*19*). The pointed appearance of the male-sterile panicle results from a longer rachis and shorter rachis branches toward the tip than is common in the normal panicle. This distinction is less apparent in many stocks with panicles of other shapes. Otherwise the phenotypes are similar in appearance, except for more side branching on male-sterile plants because of incomplete fruiting in the main head (fig. 2). Wind and insect pollinations result in producing seed in unbagged male-sterile panicles, but the amount produced varies considerably.

AWNS

In recording the results of this investigation, long awns were considered recessive. Most of the populations segregated in the ratio of 3 absent (not showing above apices of glumes) to 1 long. No attempt was made to determine the character of awns not exposed. In a few crosses, where awns were tip, short, and long, the first two classes were recorded together because they were often hard to separate, whereas phenotypes with long awns were easily recognized.

VIRESCENCE

Since nine previously reported virescents in sorghum have been designated v_1 to v_8, inclusive, and correction for the duplication extends the series to v_9, the symbols $V_{10}v_{10}$ are used for the factor pair responsible for the green and virescent phenotypes reported here. In stocks in which the character is well expressed, the virescent seedlings emerge a rich yellow to a greenish yellow, in sharp contrast to normal green seedlings, and successive leaves emerge yellow. At a later stage, which varies considerably with the stock and growing conditions but usually is the stage when three or four leaves are out, the tip of the lower leaf begins to turn green and the green color gradually extends to successive leaves up the stalk and to the bases of the blades and the sheaths. Often the panicle emerges greenish yellow, but shortly after emergence the plant becomes entirely green and cannot be distinguished from normal siblings unless new growth in the form of tillers or branches is present.

Expression of virescence is quite variable. In some homozygous virescent stocks a fairly high proportion of the seedlings die at an early stage, whereas in others many of the seedlings are so green that they cannot be satisfactorily separated from normal green seedlings. In some stocks various degrees of green striping occur. No segregations have yet been obtained to give a satisfactory explanation of these variations on the basis of definite modifiers that could be identified, and so far no emerging panicle has been observed in which the striping could be traced into the panicle distinctly enough to divide the panicle into sectors. No data are used in this paper from segregat-

FIGURE 2.—Texas Blackhull kafir, showing relative side branching on normal (*A*) and male-sterile (*B*) plants.

ing populations in which there was any question of the identity of the virescent seedlings. All virescents were staked in the late-seedling stage.

Factors for awns and male sterility have been found independent of V_2v_2, but none of the previously reported virescents has been tested directly with v_{10}. In the stocks observed, v_{10} has appeared to differ in expression from v_1 to v_9, inclusive, and is not likely to prove to be a duplicate of any of these.

CROSSES BETWEEN MALE-STERILE 1 AND 2

Through the courtesy of G. N. Rangaswami Ayyangar, seed of a stock carrying factors for male sterility in durra found at Coimbatore, India, was received at Chillicothe, Tex., in the spring of 1939. Because of short-day requirements, no plants headed in that year and only 3 seeds remained in the spring of 1940. These seeds produced 3 plants which were grown under a 9-hour photoperiod. All 3 plants were normal, and pollen from each was dusted on the stigmas of male-sterile plants of Texas Blackhull kafir. From these crosses, 15 F_1 plants, grown in a greenhouse at Washington, D. C., by J. H. Martin, produced normal anthers and set seed under bagged heads, and 28 F_1 plants, grown in the field, produced normal anthers and pollen but did not mature before frost. In 1941, small populations from seed of each of the 3 Coimbatore durra plants and from the 15 F_1 plants were grown under a 10-hour photoperiod. Each of the Coimbatore populations contained male-sterile plants, indicating that the parents were heterozygous for male sterility. All the F_2 populations except 1 contained male-sterile plants, and these populations can be classed roughly in 2 groups, which segregated in the ratios of 9 : 7 and 3 : 1 for male sterility (table 1). On the basis of independent factors for the 2 male-steriles, half of the populations should fall in each group. These F_2 populations are too small to show definitely that a given one belongs in the 9 : 7 or 3 : 1 group, but the evidence is sufficient to allow the conclusion that different genes are responsible for the 2 male-steriles. In this paper, factors for the durra normal allelomorph and male-sterile are indicated by the symbols Ms_1ms_1 and for the kafir normal allelomorph and male-sterile, by Ms_2ms_2.

TABLE 1.—*Independent segregation in the F_2 of durra male-sterile (ms_1) and kafir male-sterile (ms_2) in the cross kafir male-sterile ($Ms_1Ms_1ms_2ms_2$) × heterozygous durra male-sterile ($Ms_1ms_1Ms_2Ms_2$)*

Duplicate-factor segregation (9 : 7 ratio)				Kafir male-sterile segregation (3 : 1 ratio)			
Plot No.	Plants of indicated phenotype			Plot No.	Plants of indicated phenotype		
	Normal	Male-sterile	Total		Normal	Male-sterile	Total
	Number	*Number*	*Number*		*Number*	*Number*	*Number*
4	12	15	27	5	15	4	19
6	18	7	25	8	9	2	11
7	8	19	27	10	7	2	9
9	9	7	16	13	2	1	3
11	16	6	22	15	11	4	15
12	2	8	10	16	13	4	17
14	18	13	31	17	12	3	15
				18	8	0	8
Total	83	75	158	Total	77	20	97
Expected	89	69		Expected	73	24	

SINGLE-FACTOR INHERITANCE

Male-sterile segregates in the groups of populations shown in table 2 were consistently and, except for a few small populations, significantly short of expected numbers. This deficiency of male-sterile phenotypes has been rather general, but the cause has not been determined.

Most segregating populations had a shortage of the expected number of virescents. The original deficiency varied in different stocks, and survival from seedling stage to maturity was nearly always at least a little greater among normal siblings than among the virescents.

Segregation of awns deviated significantly from expected ratios in several groups. Some of these deviations may have resulted from the association of awns with male sterility and virescence.

LINKAGE

The linkage of factors for male sterility and awns, awns and virescent yellow, and male sterility and virescent yellow is shown in table 2. These crosses were among many made primarily to find characters associated with male sterility or to develop lines with increased seed-setting capacity on male-sterile plants, which accounts for the large number of different but related populations grown over a period of several years. All the data are from two-factor segregations. Several F_2 and backcross populations with the three-factor pairs segregating in the coupling phase were grown in 1941. A heavy infestation of sorghum midge (*Contarinia sorghicola* (Coq.)) and the occurrence of late-autumn rains, which delayed tabulations until early December, made it impossible to classify all male-sterile and normal phenotypes with certainty. Data from part of the populations were tabulated for these characters, but they appeared inconsistent with those previously accumulated and were discarded. Data from these populations were included, however, for determining linkage between awns and virescent yellow.

The cross-over percentages (table 2) appear rather variable though they are perhaps not more inconsistent than might be expected, considering the nature of the material from which the data were collected.

Weighted averages of all linkages (table 3) indicate the order of genes with approximate cross-over percentages to be as follows: ms_2 (10.9) *a* (9.1) v_{10}, or total cross-overs between ms_2 and v_{10}, about 20 percent. The weighted average of cross-over percentages where only the factor pairs Ms_2ms_2 and $V_{10}v_{10}$ were segregating is 19.3; with an expected coincidence of about 1 percent, this value is close to the sum of the other two values.

The characters stigma feathering and leaf-tip hairiness reported by Rangaswami Ayyangar and Reddy (*4*) as associated with awns were not investigated. The basal-feathered type of stigma has not been observed in any of the Chillicothe stocks, and variations in leaf-tip hairiness could not be separated into definite phenotypes. Consequently, whether factors for these characters are on the ms_2 or v_{10} side of those for awns has not been determined.

TABLE 2.—*Linkage of male sterility (Ms_2ms_2), awns (Aa), and virescent yellow ($V_{10}v_{10}$) in F_2 and backcross populations*

Year	Genes *A B*	Linkage phase	Populations	Plants in classes—				Total plants	Crossover [1]	Standard error	Recessives					
											aa			*bb*		
				AB	*Ab*	*aB*	*ab*				Percent	χ^2	*P*	Percent	χ^2	*P*
			Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Percent*	*Percent*						
1937	Ms_2A	*RS*	3	81	47	38	1	167	14.5	7.5	23.35	0.286	>0.05	28.74	1.145	>0.05
1938	do	do	3	308	153	102	0	563	[2] 10.0	4.2	18.12	14.392	<.02	27.18	1.363	>.05
1939	do	do	8	792	329	265	2	1,388	9.5	2.7	19.24	24.592	<.02	23.85	.984	>.05
1940	do	do	6	433	189	145	3	770	15.0	3.5	19.22	13.716	<.02	24.94	.002	>.05
Total or weighted average			20	1,614	718	550	6	2,888	11.0	1.8	19.25	50.888	<.02	25.07	.007	>.05
1940	Ms_2A	*CS*	15	1,080	93	56	253	1,482	11.0	.9	20.85	13.611	<.02	23.35	2.160	>.05
1941	do	*CB*	1	53	7	2	29	91	9.9	3.1	34.07	9.242	<.02	39.56	3.967	<.05
1940	AV_{10}	*RS*	2	135	54	73	0	262	[3] 13.0	6.0	27.86	1.145	>.05	20.61	2.692	>.05
1938	AV_{10}	*CS*	2	135	7	15	38	195	11.0	2.4	27.18	.436	>.05	23.08	.436	>.05
1940	do	do	3	112	5	11	30	158	10.0	2.5	25.95	.076	>.05	22.15	.684	>.05
1941 [3]	do	do	21	2,144	116	122	578	2,960	8.5	.5	23.65	2.883	>.05	23.45	3.813	>.05
Total or weighted average			26	2,391	128	148	646	3,313	9.0	.5	23.97	1.861	>.05	23.36	4.695	<.05
1941	AV_{10}	*CB*	2	178	18	21	174	391	10.0	1.5	49.87	.003	>.05	49.10	.125	>.05
1941 [3]	do	do	4	232	28	12	180	452	8.8	1.3	42.48	10.230	<.02	46.02	2.867	>.05
Total or weighted average			6	410	46	33	354	843	9.4	1.0	45.91	5.648	<.02	47.45	2.193	>.05
1937	Ms_2V_{10}	*RS*	1	105	17	38	0	160	[3] 26.0	7.3	23.75	.133	>.05	10.63	17.633	<.02
1940	do	do	1	167	75	64	3	309	21.5	5.4	21.68	1.730	>.05	25.24	.017	>.05
Total or weighted average			2	272	92	102	3	469	20.0	4.4	22.39	1.640	>.05	20.26	5.512	<.02
1940	Ms_2V_{10}	*CS*	15	897	123	95	190	1,305	19.0	1.2	21.84	6.874	<.02	23.98	.691	>.05
1941	do	*CB*	8	301	69	57	211	638	19.7	1.6	42.01	16.307	<.02	43.89	9.536	<.02

[1] In F_2 populations; estimated to nearest half percent by use of Immer's tables (*5*).
[2] Assuming one phenotype in double-recessive class.
[3] 3-point tests, but data used only for region Aa to $V_{10}v_{10}$.

TABLE 3.—*Summary of linkage data for male sterility (Ms_2ms_2), awns (Aa), and virescent yellow ($V_{10}v_{10}$)*

Linkage phase	Genes								
	Ms_2A			AV_{10}			Ms_2V_{10}		
	Populations	Total plants	Cross-over	Populations	Total plants	Cross-over	Populations	Total plants	Cross-over
	Number	*Number*	*Percent*	*Number*	*Number*	*Percent*	*Number*	*Number*	*Percent*
RS	20	2,888	11.0±1.8	2	262	13.0±6.0	2	469	20.0±4.4
CS	15	1,482	11.0±0.9	26	3,313	9.0±0.5	15	1,305	19.0±1.2
CB	1	91	9.9±3.1	6	843	9.4±1.0	8	638	19.7±1.6
Weighted [1] average			10.9			9.1			19.3

[1] Inversely as the squares of standard errors (*10, p. 325*).

SUMMARY

This paper reports a linkage group in sorghum of three pairs of genes. The phenotypes are normal and male-sterile flowers, awnless and awned lemmas, and green and virescent-yellow plants. The factor pairs are designated Ms_2ms_2, Aa, and $V_{10}v_{10}$, respectively, the symbols for awns having been used previously in other papers. The factors Ms_2 and V_{10} show complete dominance over their respective allelomorphs in the F_1 and the factor A almost complete dominance. Single-factor segregation is indicated in F_2 and backcross populations, although in most of the populations recessive classes were short of the expected numbers. The indicated order of genes with cross-over percentages is ms_2 (10.9) a (9.1) v_{10}.

Since the factors for the easily observed characters, awns and virescent plants, are linked with the factor for male sterility, a guide is provided for the removal before pollination begins of a large proportion of the normal plants from a population segregating for male sterility.

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U. S. GOVERNMENT PRINTING OFFICE: 1945